An investigation into the extraction and analysis of polychlorinated biphenyls from various foodstuffs

Mills, Andrew G.

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AN INVESTIGATION INTO THE EXTRACTION AND ANALYSIS OF POLYCHLORINATED BIPHENYLS FROM VARIOUS FOODSTUFFS

Submitted by ANDREW G. MILLS BSc MSc

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
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CONTENTS

SUMMARY

The analysis of polychlorinated biphenyls (PCBs) in a range of foodstuffs and human milk samples is reported. The results for each sample type are reported separately, along with results achieved by other researchers. The results obtained for each different sample type are compared with those previously reported in the literature, and the similarities and differences discussed.

A novel approach is described to increase sample throughput for PCB analyses over that achieved by classical laboratory techniques. The use of supercritical fluids to accelerate the extraction and clean-up of PCBs from cows milk samples is investigated. The history of the use of supercritical fluids in analytical work is reviewed, and previous research on PCB extraction by supercritical fluids is outlined. A method for the extraction of PCBs from cows milk using supercritical carbon dioxide is described. In addition, a method for the separation of the PCBs from the fats present in the milk samples is also described. A large improvement in sample throughput by using supercritical fluids is demonstrated.

A number of different quantitation and chromatographic techniques are compared for the analysis of milk samples for PCBs. Three different quantitation techniques are described for the analysis of PCBs. Six milk samples are analysed by gas chromatography-electron capture detection (GC-ECD) and gas chromatography-mass spectrometry (GC-MS). One of the quantitation techniques is used to quantify the GC-ECD data, and all three are used for the GC-MS data. The four sets of Total PCB concentrations are compared statistically to discover whether the various quantitation and chromatographic techniques give significantly different results. It is demonstrated that the use of GC-ECD gives significantly higher Total PCB results than the use of GC-MS.

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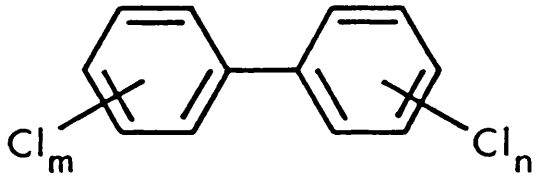
CHAPTER 1: INTRODUCTION

1.1: WHAT IS A POLYCHLORINATED BIPHENYL ?:

Polychlorinated biphenyls (PCBs) are a class of 209 discrete chemical compounds, with the general formula $C_{12}H_{10-n}Cl_n$, where n = 1 - 10. They are man-made, and consist of one to ten chlorine atoms attached to a biphenyl skeleton, Diagram 1.1.



m + n = 1 to 10

DIAGRAM 1.1: GENERAL STRUCTURE OF PCBS

From Erickson (1), pg. 1.

Each of the 209 individual PCBs is referred to as a congener. The term homologue is used for the PCBs of one particular chlorination level. The different PCBs of a given homologue are called isomers.

PCBs were known before the turn of the century, and the useful industrial properties of mixtures obtained by the chlorination of biphenyl were recognised early on in this century. The general stability and inertness of PCBs coupled with excellent dielectric properties led to their widespread commercial utility.

1.2: PRODUCTION AND PROPERTIES:

1.2.1: INDUSTRIAL PRODUCTION:

PCBs were produced industrially from 1929 up until the 1970s. The major world manufacturer was the Monsanto Corporation in the U.S.A., who produced PCBs under the trade name of Aroclor between 1930 and 1977. A list of the major world manufacturers of PCBs is shown in Table 1.1.

PRODUCER	COUNTRY	TRADEMARK OF PCB
Monsanto	U.S.A. & Great Britain	Aroclor®
Bayer	Germany	Clophen®
Prodelec	France	Phenoclor® & Pyralene®
Kanegafuchi	Japan	Kanechlor®
Mitsubishi-Monsanto	Japan	Santotherm®
Caffaro	Italy	Fenclor® & Apirolio®
Sovol	U.S.S.R.	Sovol®
Chemko	Czechoslovakia	Delor®

N.B. ® = registered trademark.

TABLE 1.1: THE WORLD'S MAJOR PRODUCERS OF PCBS

From Hutzinger, O. *et al.* (2), pg. 7.

PCBs were produced commercially, as complex mixtures, by the batch chlorination of biphenyl with chlorine gas, and the subsequent separation and purification of the desired chlorinated biphenyl fractions. The average degree of chlorination was controlled by the reaction conditions to yield the desired physical and chemical properties. At Monsanto all of the Aroclors were characterised by a four-digit number. The first two digits denoted the type of compound, with "12" indicating biphenyl, and the latter two digits gave the weight percentage of chlorine present, except Aroclor 1016. For example, Aroclor 1242 was a polychlorinated biphenyl mixture containing 42 % chlorine by weight. Other industrial manufacturers used different codes to distinguish between different PCB mixtures, Diagram 1.2 and Tables 1.2 & 1.3.

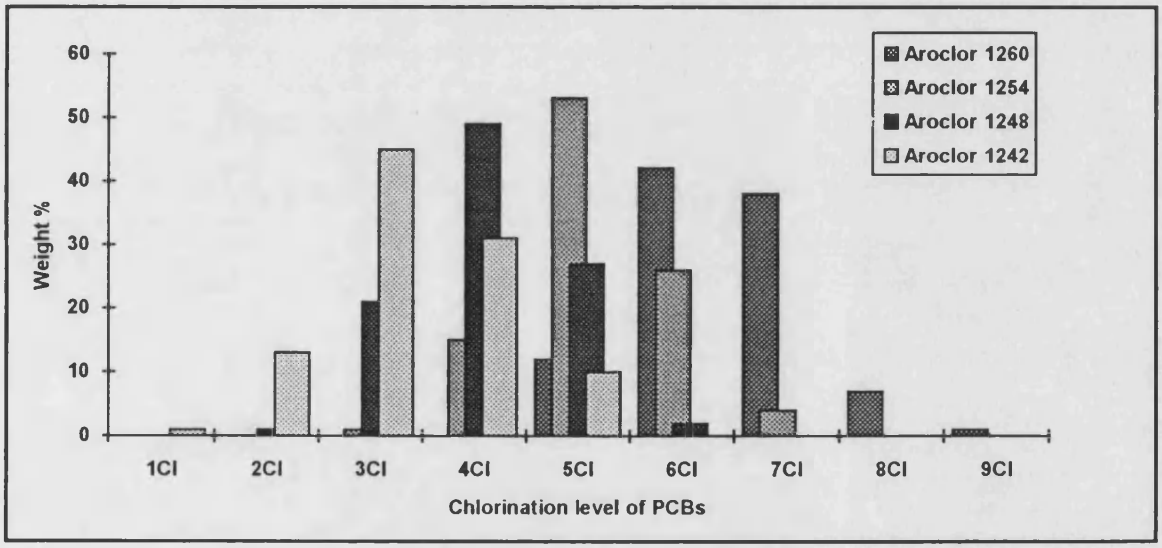


DIAGRAM 1.2: THE COMPOSITION BY WEIGHT % OF SOME AROCLORS

HOMOLOGUE (No. of Chlorines)	Average Molecular Composition (Wt. %)						
	AROCLOR No.						
	1221	1232[a]	1016	1242	1248	1254	1260
0	10						
1	50	26	2	1			
2	35	29	19	13	1		
3	4	24	57	45	22	1	
4	1	15	22	31	49	15	
5				10	27	53	12
6					2	26	42
7						4	38
8							7
9							1

a - 5% unidentified (biphenyl?)

TABLE 1.2: THE AVERAGE MOLECULAR COMPOSITION (Wt. %) OF SOME AROCLORS

From Erickson (1), pg. 19.

TRADE NAMES						Average No. of Cl per Molecule	Approx Wt. % Cl	Approx "Mol. Wt."
Aroclor[a]	Clophen[b]	Phenoclor[c]	Pyralene[c]	Kanechlor[d]	Fenclor[e]			
1221						1.15	21	193.7
1232			2,000	200		2	32-33	223.0
			1,500			2.5	38	240.3
1242, 1016	A30	DP3	3,000	300	42	3	40-42	257.5
1248	A40	DP4		400		4	48	291.9
1254	A50	DP5		500	54	5	52-54	326.4
1260	A60	DP6		600	64[f]	6-6.3	60	366.0
1262						6.8	62	388.4
					70[f]	7.7	65	419.4
1268						8.7	68	453.8
1270						9.5	70	481.4
					DK	10	71	498.6

a - Monsanto Industrial Chemicals Company, U.S.A.
b - Bayer, Germany
c - Caffaro, Italy
d - Kanegafuchi Chemical Company, Japan
e - Prodelec, France
f - The two-digit number should indicate the wt. % Cl; however this does not fit the manufacturer's specifications.

TABLE 1.3: COMPARISON OF COMMERCIAL PCB MIXTURES

From Erickson (1), pg. 18.

The major physical and chemical properties of PCB mixtures have been listed as follows: thermal stability, resistance to acids, bases, and other chemical agents, stability to conditions of oxidation and hydrolysis encountered in industrial use, low solubility in water, low flammability, high electrical resistivity, favourable dielectric constants, low vapour pressure at ambient temperature, and suitable viscosity-temperature relationships. These properties led to the extensive use of the family of PCB mixtures in a wide range of industrial applications (1). The main industrial use of PCBs was as a dielectric fluid in capacitors and transformers, accounting for up to 65 % of all PCB mixtures produced in a year for use in Japan, according to Isono (2a).

Other uses for PCBs include as industrial fluids in hydraulic systems, gas turbines, and vacuum pumps, as fire retardants, heat transfer applications, as plasticisers in adhesives, textiles, surface coatings, sealants, printing, copy paper, carbonless copy paper, and paints. In addition, some PCBs were recommended for incorporation into pesticide formulations. A number of books have appeared dealing comprehensively with the properties, chemistry and analysis of polychlorinated biphenyls, (1 - 3).

The uses of PCBs were divided into three categories by the Organisation for Economic Cooperation and Development (OECD) in 1973 (3), and these are shown in Table 1.4. In the U.S.A. in 1971 closed systems accounted for 61 % of use, with open-ended and nominally closed accounting for 26 % and 13 %, respectively.

CATEGORY	END-USE	AROCLOR 1200 SERIES
Closed		
Systems	Capacitors	16, 21, 54
	Transformers	42, 54, 60
Nominally		
Closed	Hydraulic Fluids	32-60
Systems	Heat-Transfer Fluids	42
	Lubricants	21, 42-54
Open-Ended		
Applications	Plasticiser in Rubbers	21-54, 68
	Plasticiser in Resins	48-68
	Carbonless Copy Paper	42
	Adhesives	21-54
	Wax Extenders	42, 54, 68
	Dedusting Agents	54, 60
	Inks	54

TABLE 1.4: INDUSTRIAL USES OF PCBS

From Brinkman & De Kok in Kimbrough (3), pg. 14.

PCBs can also be unintentionally produced as by-products in a wide variety of chemical processes. These include the manufacture of chlorinated benzenes, solvents, alkanes, chlorophenylsiloxane adhesives, organosilicone drugs, organic intermediates, and pigments. Some of these products contain complex mixtures of PCBs. It should also be recognised that there are by-products formed in the commercial manufacturing of PCBs. Biphenyl can be present as a by-product at more than 1 % w/w. The presence of polychlorinated dibenzofurans (PCDFs) and polychlorinated naphthalenes (PCNs) has been detected at μg/g levels. Polychlorinated quaterphenyls (PCQs), and other impurities, may be formed during the use and handling of PCBs. The presence and levels of impurities probably varies widely from batch to batch. The presence of the highly toxic PCDFs almost certainly contributes to the toxicological effects attributed to commercial PCB mixtures.

It has been estimated that 5.7×10^{11} g of PCBs were produced in total in the U.S.A., most of which is presently in service, destroyed, or otherwise unavailable. However, an estimated 1.1×10^{10} g is in the environment, most of which has been deposited in the North Atlantic Ocean, and other water sources (1).

Results from around the world show that PCBs may be considered ubiquitous pollutants. An estimation of the amount of PCBs present in the environment globally has been carried out, and the results are shown in Table 1.5. It was estimated that 3.7×10^5 tonnes of PCBs were present in the global environment in 1986. Almost all of this weight, some 3.6×10^5 tonnes, was retained in coastal sediments and open ocean (4). The oceanic water contained over 60 % of the world environmental PCB load, Table 1.5. This clearly showed that the open ocean serves as a vast reservoir and final sink of PCBs. PCBs have also been found in a whole range of animal and plant species worldwide. The environmental transport of PCBs is complex, resulting in a fairly uniform global background contamination of PCBs.

ENVIRONMENT	PCB LOAD (t)	PERCENTAGE OF PCB LOAD	PERCENTAGE OF WORLD PRODUCTION
TERRESTRIAL & COASTAL			
Air	500	0.13	
River & Lake Water	3,500	0.94	
Seawater	2,400	0.64	
Soil	2,400	0.64	
Sediment	130,000	35	
Biota	4,300	1.1	
TOTAL (A)	143,000	39	
OPEN OCEAN			
Air	790	0.21	
Seawater	230,000	61	
Sediment	110	0.03	
Biota	270	0.07	
TOTAL (B)	231,000	61	
TOTAL LOAD IN THE ENVIRONMENT (A+ B)	374,000	100	31
Degraded & Incinerated	43,000		4
Land-Stocked[a]	783,000		65
WORLD PRODUCTION	1,200,000		100

a - Still in use in electrical equipment and other products, or deposited in landfills and dumps.

TABLE 1.5: THE ESTIMATED PCB LOADS IN THE GLOBAL ENVIRONMENT

From Tanabe (4), pg. 9.

1.2.2: INDIVIDUAL PCB CONGENERS:

The use of the full chemical name to describe each individual PCB congener proved unwieldy for researchers, and led to the need for a simple shorthand nomenclature. In 1980, Ballschmiter and Zell (5) arranged the 209 PCB congeners in ascending numerical order, and assigned each congener a number from 1 to 209. One small discrepancy was corrected, and the numbering system of Ballschmiter and Zell was adopted to distinguish between individual PCB congeners. The numbers are usually referred to as "IUPAC" numbers, Appendix 1.

Mullin *et al.* (6) successfully synthesised all 209 individual PCB congeners in 1984. The synthetic methods for each PCB congener were tabulated, along with the ^{1}H-NMR data. All of the congeners were then analysed by capillary column GC-ECD, and it was found that 187 PCB congeners could be resolved using a 50 m SE-54 capillary column. This meant that only 11 pairs of congeners were not fully resolved. The relative GC retention times and response factors for each PCB congener compared to the standard, octachloronaphthalene (OCN), were also tabulated. This work made it possible for researchers to quantitate the major toxic PCB congeners which bioconcentrate in wildlife and human tissues.

1.2.3: PCBS AS A POLLUTION PROBLEM:

Swedish chemist Sören Jensen accidentally found enormous quantities of unknown substances in the tissues of pike and an eagle when he was analysing pesticides in 1966 (7). These were later identified as PCBs, which thus sparked off the great interest in environmental pollution by PCBs. The presence of PCBs in marine life and other samples in many parts of the world was soon detected. The chemical and physical stability of PCBs, the very properties that made them so attractive commercially, are responsible for the environmental contamination problems. PCBs do not readily degrade in the environment after disposal or dissemination, and are lipophilic, which means that they are persistent and tend to bioaccumulate. A study of the PCB residues in a food chain which included plankton, fish, and striped dolphins found the bioaccumulation factor from the bottom to the top of the chain was of the order of 10^{7} (8). The consumption of fish is known to

be one of the major sources of PCB contamination in humans, as well as exposure to contaminated air, water and dust. The high lipophilicity of PCBs means that they tend to accumulate in the fatty tissues.

The adverse health effects of occupational exposure to PCBs were reported as early as 1936 by Jones and Alden (9), who found evidence of chloracne amongst workers at a factory manufacturing PCBs. Workplace thresholds were set in 1936, following this report. Several further reports also catalogued the occurrence of chloracne, and other medical ailments, in workers at factories who were regularly exposed to PCBs (10, 11). The chloracne was caused by polychlorinated dibenzodioxin (PCDD) and PCDF contamination of the PCBs.

In Japan in 1968, a fatal poisoning by a brand of cooking oil took place. The cause of the problem was found to be the contamination of the oil with PCBs, PCDFs, and PCQs (1). The so-called "Yusho" incident provided information on the modes of entry of these compounds into the environment, and the routes by which they can affect man. A large number of studies on the epidemiology and health consequences of human exposure to the contaminants were carried out on the residents of Fukuoka and Nagasaki who were affected . More than 1,600 people suffered from the poisoning. The clinical and pathological symptoms of Yusho disease were characterised by chloracne, liver disfunction, and a variety of constitutional symptoms. Subsequent investigations into levels of the contaminants in blood and tissue samples from affected persons implied that the PCDFs and PCQs were responsible for the fatalities and clinical symptoms observed (12-14). The levels of PCBs in blood were found to be only twice those found in a control population, and lower than those in workers occupationally exposed to PCBs.

In 1979 a similar outbreak (called "Yu-Cheng") occurred in central Taiwan, again caused by rice oil poisoning. About 1,700 people were affected by this incident. Tests on blood and tissue samples of the victims again demonstrated that PCDFs and PCQs were almost certainly responsible for the clinical effects observed (14). A number of fires and spills have also resulted in the contamination of large numbers of people with PCBs. Incidents have been reported in the U.S.A., Canada, France, Italy, and other countries worldwide.

1.2.4: PCB REGULATION AND DISPOSAL:

The mounting evidence of the global occurrence of measureable levels of PCBs by the early 1970s began to put pressure on the regulatory authorities to do something about the problem. In mid-1971, Monsanto voluntarily ceased production destined for non-closed systems (Table 1.4), and introduced Aroclor 1016 as a more biodegradable dielectric fluid for use in capacitors. Throughout the world most producers stopped or reduced production during the 1970s, although some production is known to have continued until at least 1983. Monsanto, the major world manufacturer of PCBs, ceased all production in 1977. This followed the regulation of PCBs under the Toxic Substances Control Act (TSCA) passed by the United States Congress in 1976 (15). The TSCA regulated the manufacturing, processing, distribution, and use of PCBs, and gave the U.S. Environmental Protection Agency (EPA) the statutory powers necessary to enforce the act.

A significant drop in PCB levels in heavily polluted areas of the world was seen throughout the 1970s as a result of the ending of mass PCB production, and the imposing of acceptable levels for PCBs in the environment by various governments. However, since the early 1980s the PCB levels reported have fluctuated or declined much more slowly (16). Jones *et al.* (17) reported this type of trend for PCB levels in herbage from Great Britain between 1965 and 1989. This means that PCBs are obviously still environmentally available, and the global scale recycling of PCBs between the atmosphere and land and water surfaces has probably reached an equilibrium. Therefore, even if no more PCBs are released into the environment, an unlikely scenario given the large amounts still in use or storage coupled with mans previous pollution record, the end of the PCB pollution problem is a long way off.

The main challenge that the world now faces with PCBs is the disposal of those PCBs still in use or in storage. Large quantities of PCB-containing products such as transformer oils and capacitors have been removed from service since 1976. The problem then is what to do with these PCB products. The main method of disposal has been incineration at very high temperatures. Landfilling of materials with low levels of PCBs has also occurred. The problems of incineration

include incomplete destruction of PCBs and the formation of toxic PCDFs, PCDDs and other compounds during incineration. Stack emissions are carefully monitored to ensure that incineration does not cause the release of PCBs or other compounds at levels above stringently set limits. Landfilling can lead to the volatilisation of some PCBs, and seepage from landfill sites, particularly into underground water supplies, is also a problem requiring constant and careful monitoring. The sites for such landfilling should be fully investigated for possible geological problems before any hazardous wastes are brought to the site. Large quantities of PCBs have been disposed of by landfilling (18).

The degradation of PCBs is very slow, and their partial volatility at ambient temperatures means that they can re-enter the wider environment. PCBs from contaminated soil and water can be released into the air. They can then be transported as vapour or associated with aerosols, falling as rain or dry deposition elsewhere on the planet. PCBs can also be transported by fish and birds. These various transport processes have resulted in PCBs being present at levels in the parts per billion range in all surface soils and sediments, as well as vegetation. The higher chlorinated PCB congeners are heavier and less soluble than the lower chlorinated PCB congeners, and therefore less prone to volatilisation.

PCB regulations and disposal requirements vary from country to country, but the monitoring of PCBs goes on worldwide. There is a common interest in this, as PCBs from one area can easily pollute another if the PCBs are not carefully dealt with at source. An example of this is the pollution of the arctic environment by PCBs produced and used in North America (1).

1.2.5: PCB DEGRADATION:

There are three natural processes of degradation of environmentally dispersed PCBs: combustion, photolysis, and biodegradation. Natural combustion is very rare, and photolysis needs access to light for a sufficiently long period of time (16). Also, PCDFs and other related toxic compounds can be formed from PCBs by photolysis and combustion. Therefore, the crucial role in the environmental degradation of PCBs is played by microbial biodegradation processes. Here,

again, the story is complicated. This is because not only is biodegradation highly congener specific, but there are two contrasting and complementary types of microbial degradation.

Aerobic degradation affects the lower chlorinated PCBs, resulting in the higher chlorinated PCBs becoming more abundant. However, anaerobic degradation causes reductive dechlorination of higher chlorinated PCBs, resulting in the lower chlorinated PCBs becoming more abundant. In addition, anaerobic degradation preferentially affects the non-ortho substituted toxic PCBs.

The less chlorinated congeners that arise from anaerobic degradation can subsequently be subjected to aerobic degradation, showing that nature may be able to remove PCBs from the environment by a combination of the two degradation processes. However, anaerobic degradation only occurs for PCB levels far above those present in the environment. The subject of biodegradation has been reviewed by Abramowicz (19). The review deals in some detail with both the aerobic and anaerobic degradation of PCBs, and contains 94 references.

1.2.6: TOXICOLOGY OF PCBS:

McFarland and Clarke (20) reported that the potential toxicity of individual PCB congeners can be directly related to how closely they approach the molecular spatial configuration and distribution of forces of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (2, 3, 7, 8-TCDD). 2, 3, 7, 8-TCDD is generally considered the most potent synthetic environmental toxicant, and is regarded as a standard for comparison for other organic toxicants that are more or less isosteric, such as some PCB congeners.

The most toxicologically active PCB congeners are those having chlorine substitution at the para and at least two meta positions on the biphenyl nucleus, but no ortho substitutions. Such congeners have the possibility to assume a coplanar configuration similar to that of 2, 3, 7, 8-TCDD.

McFarland and Clarke have arranged the PCB congeners into groups on the basis of toxic potential, as judged by chemical structure, which influences the ability to stimulate the production of bioactivating enzyme systems. Congeners that demonstrate 3-methylcholanthrene-type (3-MC-

type) and mixed function oxidase-type (MFO-type) induction have the greatest toxic potential, i.e. congeners that can adopt the coplanar configuration or closely resemble it. The larger group of phenobarbitol-type (PB -type) inducers have considerably less potential for contributing to any toxic effects. Weak inducers and non-inducing congeners have the least potential for toxicity.

McFarland and Clarke have suggested that priority groupings for specific PCB congeners having potential environmental significance can be made. These are based upon three factors:
a. Potential for toxicity; b. Frequency of occurrence in environmental samples; c. Relative abundance in animal tissues.

These priority groupings are shown in Table 1.6. The potentially most toxic congeners are in Group No. 1, with potential toxicity decreasing through to Group No. 4.

	PRIORITY GROUPINGS			
	No. 1	No. 2	No. 3	No.4
PCB No.	77	87	18	37
	126	99	44	81
	169	101	49	114
	105	153	52	119
	118	180	70	123
	128	183	74	157
	138	194	151	158
	156		177	167
	170		187	168
			201	189

TABLE 1.6: SUGGESTED PRIORITY GROUPINGS OF TOXIC PCB CONGENERS

From McFarland and Clarke (20).

An analysis for specific PCB congeners in environmental samples allows a more meaningful assessment for possible adverse biological effects by focussing only on those congeners that are part of the priority groupings.

1.2.7: METABOLISM OF PCBS:

The major PCB metabolites in higher animals are mono- and dihydroxychlorobiphenyls and their conjugates. Methylthio- and methylsulphone PCB derivatives have also been identified in environmental matrices. The *in vivo* PCB metabolites are shown in Diagram 1.3.

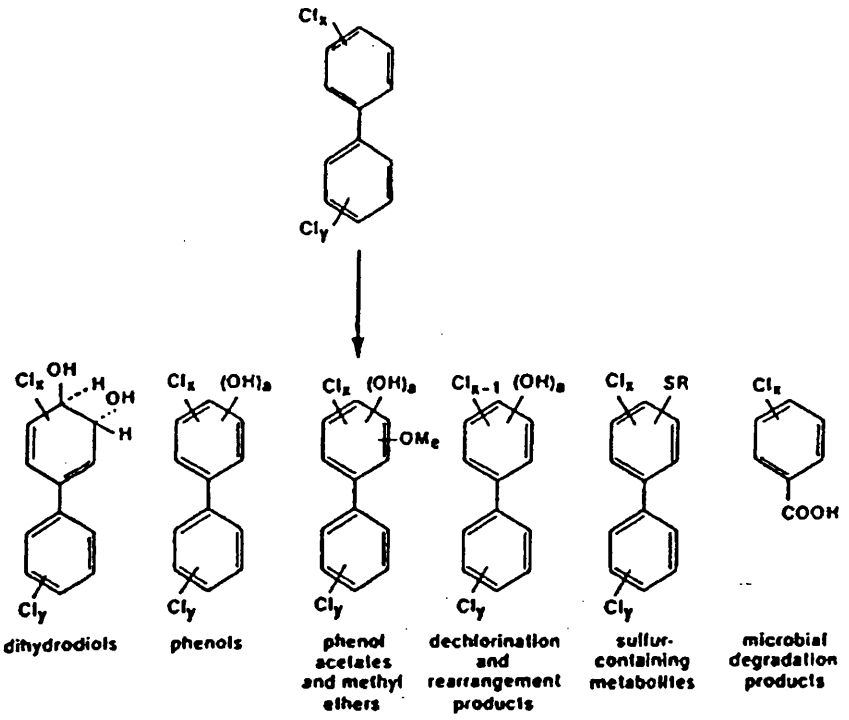


DIAGRAM 1.3: COMMON *IN VIVO* PCB METABOLITES

From Erickson (1), pg. 40.

The metabolic rates of PCB congeners are both isomer- and homolog-dependent. The higher the homolog, the slower the metabolism. Biological degradation does not significantly reduce the total environmental burden, although it does have an effect on the patterns of PCB congeners observed passing through the food chain.

1.3: GENERAL BACKGROUND LEVELS OF PCBS:

There have been a huge number of reports on the levels of PCBs found in environmental and biological samples from all around the world. The first investigations of PCB residues were carried out shortly after the work of Jensen (7) was published in 1966. A number of researchers found PCBs in marine species and birds in Europe and the U.S.A. (21-24). A review of the earliest reports on PCBs found in the environment was written by Edwards (25) in 1971. PCB levels in almost every part of the world have been established over the last 25 years.

PCBs have been found across the globe, with higher concentrations being found in more heavily industrialised areas. The highest levels of PCBs have been found either near where the production of PCBs has taken place, or where an accidental release of PCBs has occurred. The Hudson River Basin in New York State, U.S.A. is an example of an area that has been polluted by PCB production plants (1).

Des Rosiers (26) reported the analyses of chlorinated combustion products, i.e. PCBs, PCDFs, and PCDDs from fires involving PCB transformers and capacitors in the U.S.A. It was found that PCDFs were formed during these fires, and that PCBs were also detected in soot and other samples taken from near the fires. PCDDs were only formed if chlorobenzenes were also present in the transformer fluids. The number of reported PCB transformer fire incidents continues to grow in the United States and in Europe.

The global background levels of PCBs in various parts of the environment have been investigated by a range of researchers. PCBs have been detected in biological and environmental specimens from diverse locations. The PCBs have gained entry to the environment as the result of leaching from landfills, from disposal into sewers, rivers, and coastal waters, and from transport into the atmosphere. Since the 1970s, a large number of reports have found PCBs to be present in air samples, water, sediments, and soil samples.

Bush *et al.* (27) reported the levels of 74 individual PCB congeners in a variety of water samples, as well as Caddisfly larvae, from the Upper Hudson River area in the U.S.A. A capillary

GC-ECD was used for the analyses, and a mixture of Aroclors was used for the quantitation. Total PCB concentrations of between 100 ng/l and 586 ng/l were reported for water collected from three sites in July and August 1983.

Badsha and Eduljee (28), in 1986, reported the levels of PCBs in soil and grass samples from different sites in Great Britain. A packed column GC-ECD system was used, and a mixture of Aroclors were used as standards. A significant difference between results for urban and industrial areas, and those for rural areas was reported. The levels of PCBs found in rural areas were much lower than those found in the other areas. Soil samples from industrial locations were found to contain an average of 41 ng/g dry weight of Total PCBs, and soil samples from urban areas contained an average of 43 ng/g dry weight of PCBs, whereas soil samples from rural areas contained an average of only 8 ng/g dry weight of PCBs. A grass sample from an urban area contained 40 ng/g dry weight of PCBs, while grass samples from rural areas contained an average of only 9 ng/g dry weight of PCBs. These results for environmental PCB levels in Great Britain were shown to fall within the range of values reported for other countries, e.g. U.S.A., Sweden, Japan and the Netherlands.

A report to the Welsh Office in 1994 (29) gave concentrations of PCBs, PCDFs, and PCDDs in soil and air samples collected near Pontypool, Wales. A total of 27 soil samples were analysed for the 7 individual congeners PCBs No. 28, 52, 101, 118, 138, 153, and 180. A Total PCB concentration was also calculated. 9 of the 27 soil samples were found to contain Total PCB concentrations of more than 200 ng/g, with a highest reported value of 1,130 ng/g. All of these samples were collected from inside or very near to an industrial incineration plant. The other 18 soil samples had Total PCB concentrations of less than 200 ng/g.

The report (29) also stated that typical PCB concentrations in urban air in Great Britain were likely to be between 0.3 ng/m^3 and 1.0 ng/m^3. Air samples were collected from three distinct sites. One site was at the incineration plant, another next to the incineration plant, and the third was further away from the plant. The mean Total PCB concentrations for the three sites were 15.1

ng/m^3, 5.8 ng/m^3, and 1.0 ng/m^3, respectively. The first two of these results were well outside the typical Total PCB concentrations of between 0.3 ng/m^3 and 1.0 ng/m^3.

The soil and air results in this report (29) implied that PCBs were being emitted from the industrial incineration plant.

Nakano *et al.* (30) reported an average concentration of 2.8 ng/m^3 of PCBs in samples of urban air from around the city of Kobe in Japan. A capillary GC-MS with selected ion monitoring was used for the analyses. A mixture of different Kanechlors was used as the quantitation standard.

Picer and Picer (31) reported the levels of PCBs in sediment samples from the coastal waters of the Eastern Adriatic. A GC-ECD was used for the analysis. An average concentration of Total PCBs of 23.2 ng/g dry weight was reported for 41 sediment samples collected between 1976 and 1990.

Chevreuil and Granier (32) investigated the pollution by PCBs of drinking water in the Paris area of France. The samples were analysed using a capillary column GC, and quantification was done by comparison to a mixture of Aroclors 1242, 1254, and 1268. Monthly samples were taken from household taps at four locations over an 18 month period. Samples were also taken from a filtration plant, and bottled water was also analysed. The average Total PCB concentrations for the four taps fell between 108 ng/l and 115 ng/l, while water from the filtration plant had an average Total PCB concentration of 79 ng/l. Three types of bottled water had average Total PCB concentrations between 11 ng/l and 21 ng/l. 40 % of the tap water samples showed Total PCB concentrations higher than the 100 ng/l standard set by the European Economic Community (EEC), some by a large amount, with a highest recorded level of 320 ng/l. It was reported that the contamination was probably caused by seepages from one or more of the municipal dumps in the Paris area. These results also showed that approximately 65 % of PCBs were not being removed from the water by the filtration plant.

1.3.1: LEVELS OF PCBS IN PLANTS AND ANIMALS:

Since the initial discovery of PCB contamination in bird tissues in 1966 (7), PCBs have been found in nearly all marine plant and animal specimens, fish, mammals, birds, bird eggs, and humans.

Discussions on the occurrence of PCBs in fish oils, eggs, and cows milk samples from around the world can be found in later chapters.

1.3.2: LEVELS OF PCBS IN HUMANS:

A number of researchers have reported levels of PCB contamination in a range of samples from humans. For example, Jan and Tratnik (33) found higher levels of PCBs in the blood of residents living near the River Krupa in Slovenia than in people living further away from the river. The river had been contaminated by a plant using various technical PCBs. The river was used by local residents for washing and bathing, and this was suspected to be the cause of the higher PCB levels found in blood samples from these residents. A GC-ECD with a capillary column was used for the determinations, and a mixture of Aroclors was used for the quantitation procedure. An average concentration of 155 ng/g of Total PCBs was found in people living near the river. In contrast, an average concentration of only 11 ng/g was found in people living 1 to 3 kilometres from the river, and one of only 5 ng/g for people from different parts of Slovenia.

Mes *et al.* (34) reported the presence of PCBs and other organochlorine compounds in the adipose tissue of the Canadian population. The PCBs were analysed on a packed column GC-ECD, with Aroclor 1260 as the quantitation standard. The identities of the PCBs were confirmed using a capillary column GC-MS with selected ion monitoring. 108 samples were analysed from all areas of Canada, and PCBs were found in all of them at a mean concentration of 410 ng/g of wet tissue. This value was compared with values of 1,750 ng/g for an Italian population (Focardi *et al.*, 35), 800 ng/g for a British population (Abbott *et al.*, 36), and 1,680 ng/g for a Spanish population (Camps *et al.*, 37). The Canadian result was lower than the rest, but differences in extraction and quantitation procedures probably accounted for the apparent discrepancies in the

results. Some differences in the PCB levels for different regions of Canada were seen, and increased PCB levels were seen in older members of the studied population. No significant difference in the levels of PCBs was found between the sexes, although the mean value for men was higher than for women.

Burse *et al.* (38) reported the levels of PCBs in the serum of some residents of Pennsylvania, U.S.A., and their dogs, who lived near a factory which worked on electric car repairs. The contamination of soil on and near the site with PCBs had been documented. A packed column GC-ECD was used for the quantitation of the PCBs, while capillary column GC-ECD and GC-MS were also used. Average Total PCB concentrations of 5.55 ppb and 6.22 ppb were found for the dog serum and human serum, respectively. The serum samples were quantified using standard solutions of Aroclors 1260 and 1268.

Schecter *et al.* (39) reported levels of PCBs, as well as dioxins, dibenzofurans, and organochlorine pesticides (OCPs), in human milk and blood from a number of different countries worldwide. The total PCB levels were found to be highest in Siberia, Germany, and the U.S.A. All three areas were examples of heavily industrialised economies with a history of PCB production. Slightly lower PCB levels were found at two sites in southern Vietnam which, although not heavily industrialised, were contaminated during the war in the 1960s. The lowest PCB levels were found in Thailand, Cambodia, and northern Vietnam, which were examples of less industrialised Asian countries. All of the results were reported on a lipid basis.

A discussion of the occurrence of PCBs in human breast milk samples from around the world can be found in Chapter 6.

1.3.3: AVERAGE DIETARY INTAKE OF PCBS:

Matsumoto *et al.* (40) calculated the average daily intake of PCBs in the total diet of people from Osaka in Japan. A range of basic dietary foods were cooked and then analysed for PCBs. These included rice, fruit, vegetables, fish, meat, eggs, and dairy products. Daily dietary intakes of between 1,100 ng and 4,300 ng were calculated for the years 1977-1982 and 1985, with an overall

average of 2,430 ng +/-1,200 ng. The daily intakes found were the same as those reported for the whole of Japan between 1977 and 1982. The values were also similar to those reported by Gartrell *et al.* (41, 42) for the daily dietary intake in the U.S.A. between 1978 and 1980.

Mes *et al.* (43) investigated the levels of some PCB congeners in fatty foods of the Canadian diet. A total of 93 fatty food composites from the cities of Ottawa and Halifax were analysed for 34 selected PCB congeners. Capillary column GC-ECD and GC-MS were used for the analyses, and a solution containing all of the 34 PCB congeners under investigation was used for quantitation. The sum of the PCB congeners gave residue levels on a wet weight basis of between 0.03 ng/g and 1.98 ng/g for all the different foods, except for fish and fish products. The fish and fish products had residue levels of between 3.2 ng/g and 21.0 ng/g. The residue levels for whole milk and eggs were 0.2 ng/g and 0.7 ng/g, respectively. All the PCB residue levels found were well below the Canadian government's guidelines for PCBs in foodstuffs.

Mes and Weber (44) reported the levels of non-orthochlorine substituted coplanar PCB congeners (PCBs No. 77, 126, 169) in Canadian fatty foods, and other samples, using a method first reported by Tanabe *et al.* (45). The paper of Mes and Weber (44) followed on from the work of Mes *et al.* (43). The same extraction methodology was used, and, in addition, a charcoal column was used to separate the coplanar PCBs from the other PCB congeners. This was followed by a sulphuric acid clean-up step before analysis. Capillary column GC-ECD and GC-MS were used for the analyses, and a standard containing the three PCB congeners of interest was used for quantitation. The three coplanar PCB congeners were found in all of the foodstuffs analysed. The sum of the three congeners found in the samples ranged between 2 pg/g and 44 pg/g on a wet weight basis. The highest PCB concentration, 44 pg/g, was again found in fish samples, with a concentration of 3 pg/g found in eggs.

Pastor *et al.* (46) reported the levels of coplanar PCB congeners (PCBs No. 77, 81, 126, 169) in a sample of herring oil from Spain. A sulphuric acid treatment, followed by a Hypercarb LC column was used to separate the coplanar PCBs from the other PCB congeners. Capillary GC-ECD with two different columns was used for quantitation, and capillary column GC-MS was used

for confirmation of peak identity. A standard containing the four congeners of interest was used for quantitation. The amounts of coplanar PCBs found in the herring oil sample were 3 ng/g, 2 ng/g, 2 ng/g, and 2 ng/g for PCBs No. 77, 81, 126, and 169, respectively. This was much higher than the amount found in whole fish by Mes and Weber (44), but this would be expected as PCBs tend to accumulate in the fatty or oily parts of an organism.

When analyses of PCBs are performed, it must be remembered that congener compositions found in samples from different areas of the environment, and biological materials are influenced by the fact that each congener differs from the others in its properties. Consequently, the abundances of individual congeners in environmental and biological samples do differ from those in the industrial products from which the contamination originated. Moreover, contamination may originate from more than one source, and from PCBs present unintentionally as by-products. Environmental samples which have been contaminated by commercial PCB mixtures or other PCB sources may also contain other groups of chlorinated compounds, e.g. PCDFs, PCQs, PCDDs, etc. These compounds may be able to interfere with the determination of PCBs, leading to the misinterpretation of experimental results. Each group of compounds might be present as a complex mixture of many individual compounds. They can be persistent, toxic, and some have the same mechanism of action as the planar PCBs.

1.4: EXPERIMENTAL METHODOLOGY FOR THE ANALYSIS OF PCBS:

The analysis of trace levels of PCBs, and other pollutants, in environmental and biological samples involves multiple extraction and clean-up stages, followed by a chromatographic step. The first report to discuss the difficulties of successfully extracting and analysing PCBs in complex organic matrices was the IUPAC Commission report in 1967 (47). This report outlined some of the practical difficulties involved in obtaining a successful analytical result for a number of different pesticides and PCBs. Subsequently, a large number of researchers have made

important contributions to the successful extraction and analysis of PCBs from a wide range of matrices. Some of these reports are discussed below.

1.4.1: THE EXTRACTION OF PCBS:

The first step to achieve a successful analysis is to quantitatively extract the compounds of interest from the sample matrix into an organic solvent, ready for the rest of the analysis. The initial extraction of PCBs has often been carried out with a Soxhlet apparatus, e.g. Koeman *et al.* (23), which was already in widespread laboratory use for extracting other compounds, before being used for PCBs. The extraction of PCBs from environmental matrices has also been achieved by shaking with organic solvents (22), ultrasonication (33), or by using a blender or mixer (48). Treatment of samples with strong acids or bases (14), which do not destroy PCBs, has also been used. All of these extraction procedures were adapted from previous work done to extract other organic compounds, e.g. pesticides, from a wide range of sample matrices.

The proven reliability and reproducability of the Soxhlet extraction method led to its widespread use for the extraction of trace organics, including PCBs, from environmental samples. A wide range of organic solvents, including mixed solvents, have been used by different researchers to achieve the desired extraction. Soxhlet extraction remains one of the methods most widely used by analytical chemists up until the present day.

The most recent technique which has been successfully used to extract trace organics from different matrices is supercritical fluid extraction (SFE), a full review of which appears in Chapter 7.

The exact extraction methodology used for a given sample will be highly dependent upon the nature of the matrix under investigation. PCBs, which are nonpolar, are highly soluble in nonpolar solvents such as hexane, and also in lipids. Therefore, the extraction of PCBs from lipids is more difficult than extraction from, for example, water. Additionally, the sample for extraction may need to be mixed, crushed, or otherwise treated before an extraction, to ensure that the extracting solvent comes into contact with the entire sample. If this is not done, PCBs may remain still

trapped in the sample matrix after an extraction. This would then lead to erroneous results for that particular sample. This underlines the care and attention to detail needed to successfully extract trace organics, such as PCBs, from different matrices.

1.4.2: THE CLEAN-UP OF PCB EXTRACTS:

The extraction step is almost always followed by a clean-up step, which removes other compounds present in the extract that may interfere with the successful analysis of PCBs. The extent of the clean-up that is needed varies with the chromatographic technique that is to be used. The highly selective GC-MS does not require the removal of other trace organics before analysis, but is highly susceptible to even very small quantities of lipid present in a sample. By contrast, GC-ECD requires that all other organic compounds are removed from a sample prior to analysis for PCBs, but is impervious to small amounts of lipids in the sample. The clean-up of environmental samples is usually achieved with column chromatography. The early researchers used Florisil (23), silica (49), or alumina (21) columns to separate the organic compounds from co-extractants such as lipids.

The use of Florisil column chromatography as a clean-up step was first demonstrated and validated for the analysis of a range of organochlorine pesticides in fruit and vegetables by Mills *et al.* in 1963 (50). The Florisil used had been pre-activated, and was subsequently activated at 130 ^{o}C for 5 hours. Recoveries of over 90 % were reported for the pesticides, the analyses having been carried out on a GC with a coulometric detector. Florisil column chromatography has since been widely used as part of the clean-up procedure for PCBs.

Although the clean-up steps already discussed ensured that the PCBs were now comparatively free of lipid material, a host of other organic compounds were still present along with the compounds of interest. Armour and Burke (51) were the first researchers to try and separate the polychlorinated biphenyls from pesticides prior to chromatographic analysis. Previous papers on PCB analysis had relied on the gas chromatography step to separate the PCBs from the pesticides. This approach proved problematic because of the similarity of retention times of PCB congeners and some chlorinated pesticides on the GC columns commonly used. Armour and Burke

saw that a way of avoiding this confusion over the interpretation of GC data was to separate the PCBs and the pesticides before the GC step. After a Florisil clean-up, column chromatography on silicic acid-Celite was used to separate PCBs from DDT, metabolites of DDT, and other common chlorinated pesticides. Complete separation of PCBs (as Aroclors) from all of the pesticides tried, except aldrin, was reported. This ensured that the subsequent GC analysis for PCBs would not be adversely affected by the presence of other compounds in the final extract.

Stalling *et al.* (52) used a gel permeation chromatography (GPC) method instead of a Florisil column to separate PCBs and pesticides from the extracted lipids of fish extracts. Clean-up using Florisil or alumina columns took advantage of the differences in polarity between lipids and the organic compounds of interest to achieve a separation. However, these differences in polarity were often small and, consequently, the clean-up was not always effective (53). Stalling *et al.* (52) used a Bio Beads S-X2 GPC column, with cyclohexane as the eluting solvent. This gave quantitative recoveries of the trace organics, and less than 0.5 % of the lipids were extracted along with these organic compounds. When the GPC method was used in conjunction with the method of Armour and Burke (51) described above, PCB extracts free of lipids and pesticides were obtained. Hopper (54) also used a similar GPC method to clean-up samples of butterfat, corn oil, and cod liver oil. Size exclusion chromatography (SEC) has also been used, coupled with GC, as reported by Grob & Kälin (55).

In addition, HPLC has been used as a clean-up step for PCBs. Seymour *et al.* (56) used a 300 x 7.5 mm i.d. Polymer PLRP-S column to separate PCBs and organochlorine pesticides from lipids. A mobile phase of 80 % heptane and 20 % propan-2-ol at a flow rate of 2.3 ml/min was used. A lipid sample, a mixture of triglycerides called Witepsol H15, was mixed with a sample of PCBs, a mixture of Aroclors 1242 and 1260. 2.0 ml of the mixture was injected onto the column, and monitored on a refractive index detector. The lipids eluted between 4 and 6 minutes, with the PCBs eluting between 6 and 12 minutes. This method was found to have several advantages over the traditional column chromatography methods. These included low solvent consumption and a high lipid capacity. Continuous monitoring of the eluent to determine exactly when the species of

interest should be collected was possible, but high concentrations of the Aroclors were used (up to 100 ppm) for this work.

A review of the different extraction and clean-up methods used to achieve the analysis of PCBs in lipid-containing foodstuffs has been written by Liem *et al.* (57). The use of Soxhlet extraction and partitioning of samples with organic solvents are covered. Clean-up procedures using column chromatography, HPLC, and supercritical fluid extraction (SFE) are all reviewed. A number of different chromatographic and spectroscopic techniques of analysis are also detailed. The review contains 172 references.

Supercritical fluid extraction (SFE) has been also been successfully used to clean-up extracts from different matrices, and a full review appears in Chapter 7.

1.4.3: THE ANALYTICAL DETERMINATION OF PCBS:

The early researchers used packed column GC with a flame ionisation detector (FID) (21) or an electron capture detector (ECD) (23) for the analysis of PCBs. GC linked to a mass spectrometer (MS) was also used for confirmation of peak identities (25). The use of both GC-ECD and GC-MS to analyse for PCBs is reviewed below.

Three distinct types of standards have been used to quantify PCBs by GC-ECD or GC-MS. The initial determinations used commercial formulations such as Aroclor 1242, 1254, and 1260, or a combination of these standards. A mixture of single congeners, containing one congener for each level of chlorination has also been used. Finally, a mixture of selected congeners has been used, the congeners having been chosen on the basis of persistence and toxicity. The choice of standard is based on a number of factors including the type of sample, the availability of reference compounds, and the objective of the determination. The use of different quantitation standards is discussed below.

1.4.3.1: The Analytical Determination Of PCBs By GC-ECD:

Most of the early analyses of PCBs were carried out by packed column GC-ECD. In the majority of cases, the quantitation of PCBs by packed column GC-ECD was done by comparison of the experimental chromatograms obtained with chromatograms of Aroclor solutions, or other industrial PCB mixtures, of known concentrations (58). The total areas under the peaks of interest were compared for the sample and standard chromatograms, and a Total PCB concentration in the sample was then reported.

Real samples may contain a combination of Aroclors or partially degraded Aroclors and, therefore, more sophisticated quantitation methods were required. The most widely used method was introduced by Webb and McCall (59), who described a quantitation method for the packed column GC-ECD analysis of PCBs which used Aroclors 1221, 1232, 1242, 1248, 1254, and 1260 as standards. Each of the Aroclors was separately analysed by both packed column GC-MS and packed column GC with an electrolytic conductivity detector. The GC-MS results were used to find out the composition, by number of chlorine atoms, of each chromatographic peak. Many of the GC peaks corresponded to a number of congeners of different chlorination level. The relative amounts of congeners of different chlorination level were calculated from the GC-MS data. An electrolytic conductivity detector was used because it responded linearly to chlorine. Therefore, the peak areas obtained by using this detector were used to calculate the percentage of the total Aroclor weight represented by each GC peak, with p,p'-DDE used as an internal standard. These results could then be used to calculate the response factors for each peak in a standard of known concentration on any GC-ECD. Each peak in a chromatogram was identified by calculating the retention time relative to the p,p'-DDE. This work led them to recommend the use of Aroclors 1242, 1254, and 1260, as appropriate, for quantifying PCBs by packed column GC-ECD.

The advent of capillary column GC allowed a great deal more information to be gathered from analysing extracts, but also increased the difficulty of quantitation because of the greater numbers of peaks now present in the chromatograms. Similar approaches to the work of Webb and McCall (59) were used for capillary column GC-ECD. One such example of this approach

was the work of Albro *et al.* (60). They determined the relative molar percentages of about 100 different PCB congeners in Aroclors 1248, 1254, and 1260. Mixtures of these Aroclors could then be used to determine capillary column GC-ECD response factors.

A slight variation on this method was also used to successfully quantify PCBs. This involved using statistical methods to quantify the PCBs in samples analysed by capillary column GC-ECD. Dunn III *et al.* (61) used pattern recognition techniques to analyse PCBs in environmental samples, with Aroclors 1242, 1248, 1254, and 1260 used as quantitation standards. A sample of used transformer oil was quantified by two different pattern recognition techniques.

The use of capillary columns meant that it was now possible for isomer-specific PCB analyses of samples to be carried out. Bush *et al.* (62) assigned 72 peaks in a mixture of Aroclors 1221, 1016, 1254, and 1260. These assignments led to most of the PCB peaks in a number of environmental samples being positively identified.

Zell and Ballschmiter (63) established which congeners were indicative of metabolism, or other degradation processes for different types of environmental samples. This allowed attention to be focussed on the individual PCB congeners of interest in particular samples.

Following the work of Mullin *et al.* (6), who had successfully synthesised all of the 209 individual PCB congeners, Safe *et al.* (64) used these results to analyse a human milk extract for 92 individual PCB congeners. PCBs No. 28, 33, 52, 99, 101, 110, 118, 138, 153, 156, 170, and 180 were among the congeners present at the highest concentrations in the human milk extract.

Tuinstra *et al.* (65) determined the concentrations of 30 individual PCB congeners in cattle feed samples. PCBs No. 52, 101, 138, 153, and 180 were among the congeners present at the highest concentrations. This led to the same group selecting 6 individual PCB congeners for use in quantitation work, these congeners being PCBs No. 28, 52, 101, 138, 153, 180. These 6 PCB congeners had been found in a wide range of samples, and were present at the highest concentrations. These 6 PCB congeners were used for regulatory analyses, where the full quantitation of PCBs present below the regulatory cut-off level was of no interest.

Mes & Marchand (66) analysed a total of 29 individual PCB congeners in samples of human milk and monkey milk by capillary column GC-ECD. The selection of the congeners was based on the most prevalent PCB congeners in human milk reported by Safe *et al.* (64). Mes & Marchand found that PCBs No. 74, 99, 118, 138, 153, and 180 were the main PCB congeners found in both the human milk and monkey milk samples.

Mes *et al.* (43) analysed 34 individual PCB congeners in a total of 93 fatty food samples. The samples were analysed by capillary column GC-ECD, and capillary column GC-MS was used for confirmation of peak identities. The results for all 34 PCB congeners were reported in the paper, and PCBs No. 52, 138, 153, and 180 were, again, among the congeners present at the highest concentrations.

The prevalence of certain individual PCB congeners in environmental samples led to several countries naming some individual congeners in PCB legislation. For example, 6 individual congeners, PCBs No. 28, 52, 101, 138, 153, and 180, are now used in all German legislation on permissable PCB levels in different samples. The Netherlands also uses the same 6 congeners, as well as PCB No. 118, in all PCB legislation.

Capillary column GC-ECD also allowed researchers to analyse samples for the planar PCB congeners which, although present at lower concentrations than many other PCB congeners, were of interest because of the high toxicity they exhibit. Mes and Weber (44) analysed the levels of PCBs No. 77, 126, and 169 in a number of fatty food samples. All three congeners were found in all of the samples analysed, and capillary column GC-MS was used to confirm peak identities.

1.4.3.2: The Analytical Determination Of PCBs By GC-MS:

Bonelli (67) reported the first quantitation of PCBs by GC-MS, which was achieved by adding together the areas under the peaks in the same way as previously used with GC-ECD (58). A mixture of Aroclor 1254 and chlorinated pesticides and naphthalenes was prepared, and run on a packed column GC-MS. Peaks were identified using their recorded retention times and mass

spectra. Quantitation was achieved by comparing the area under one or more of the PCB peaks to the area of a known amount of a standard.

A statistical approach to quantitation by capillary column GC-MS using standard areas for 40 peaks in Aroclors 1242, 1248, 1254, and 1260 to obtain an "Aroclor" concentration was introduced by Liu *et al.* (68). The statistical approach was applied to a number of spiked sediment samples, and was found to give PCB concentration results which were within 10 % of the known correct values.

The response factors (RRFs) for 77 individual PCB congeners, relative to $C_{12}{}^2H_6Cl_4$, were calculated for both quadrupole and magnetic sector mass spectrometers by Erickson *et al.* (69). The response factors for congeners within a specific homologue or degree of chlorination were averaged. The individual congener that exhibited a response factor nearest to the average response factor was selected as a calibration standard for each homologue. A calibration standard containing 11 individual PCB congeners, two monochlorobiphenyls and one each from the other homologues, was then prepared. The 11 individual congeners selected were PCBs No. 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, and 209. All of the sample peak areas recorded were totalled for each homologue, and the sum was quantified by comparison with the peak area for a known concentration of the relevant PCB congener. This allowed a PCB concentration by homologue to be reported for the analysed sample.

Capillary column GC-MS has also been used to analyse individual PCB congeners in the same way as capillary column GC-ECD. For example, Steinwandter (70) used capillary column GC-MS to analyse 20 individual PCB congeners in human milk samples. The 20 congeners consisted of 5 non-ortho substituted PCBs and 15 mono-ortho substituted PCBs.

Heidmann (71) analysed a set of 38 PCB congeners, from tetrachloro to octachlorobiphenyls, in animal tissue samples by capillary column GC-MS. PCBs No. 118, 138, 153, and 180 were among the congeners found to be present at the highest concentrations. The identities of the peaks were not all unambiguously confirmed.

In addition, the experimental percentage recoveries of PCBs have been calculated by capillary column GC-MS using surrogates. Erickson *et al.* (69) used a set of 4 ^{13}C-labelled PCB congeners to calculate percentage recoveries. The 4 congeners used were each from different homologues, i.e. a monochloro, a tetrachloro, an octachloro, and a decachlorobiphenyl.

The 4 congeners were (1', 2', 3', 4', 5', 6'-$^{13}C_6$)-4-chlorobiphenyl, ($^{13}C_{12}$)-3, 3', 4, 4'-tetrachlorobiphenyl, ($^{13}C_{12}$)-2, 2', 3, 3', 5, 5', 6, 6'-octachlorobiphenyl, and ($^{13}C_{12}$)decachlorobiphenyl, respectively. Knowledge of the experimental percentage recovery is useful in monitoring extraction and clean-up performances. The final reported concentrations may be corrected for recovery, or the PCB concentration found and the percentage recovery reported separately.

1.4.3.3: Comparison Of The Analytical Determination Of PCBs By GC-ECD And GC-MS:

Pavoni *et al.* (72) compared the quantitation of PCBs in sediments, algae, mussels, and worms by capillary column GC-ECD and GC-MS, in the SIM mode, using Aroclor mixtures as standards. Two different Aroclor mixtures were used for different samples, a 1:1 mixture of Aroclors 1242 and 1254 and a 1:1 mixture of Aroclors 1254 and 1260. In addition, the quantitation of the PCBs in the samples by GC-MS was also compared using two different standards. The two different standards were the appropriate Aroclor mixture and a 7 congener mixture of PCBs, with one congener for each level of chlorination from monochlorinated to heptachlorinated.

The quantitation by GC-ECD was found to be more precise than that by GC-MS. The precision for the GC-ECD ranged from 3 % to 7 %. The small level of imprecision was caused by incomplete peak resolution, and not uncertainties in peak area measurements. The same precision was found over the whole chromatogram. In contrast, the precision for the GC-MS ranged from 10 % to 18 %. The precision for the earlier peaks in a chromatogram was better than for the later peaks, which were smaller and more difficult to integrate. The main reason for the difference in precision was the greater sensitivity of the GC-ECD over the GC-MS. In addition, the sensitivity

of the GC-ECD increased with an increase in the level of PCB chlorination, while the sensitivity of the GC-MS decreased. This was because the ECD response increased with increasing chlorine content, while the MS response decreased with increasing molecular weight.

There was a small increase in precision in the results obtained by using the 7 congener mixture for quantitation on the GC-MS instead of the Aroclor mixture. However, there was no significant difference in the actual concentrations obtained by using the two different quantitation standards.

The concentrations obtained by GC-ECD were systematically higher than those obtained by GC-MS using either quantitation standard. This was because PCB analyses carried out by GC-ECD, even though they are preceded by steps to minimise interferences, can always be affected by over-estimation due to the inclusion of extraneous peaks. These may be due to pesticides or other chlorinated hydrocarbons. It was reported that the GC-MS data in this study was more accurate than the GC-ECD data by about 10 %.

Pavoni *et al.* (72) concluded that analyses with GC-ECD were easier to perform and cheaper, and should be recommended when large numbers of samples were to be analysed. In contrast, sample preparation and instrument tuning had to be done with greater care to achieve accurate results by GC-MS. This makes GC-MS analysis more difficult to perform, and time-consuming. However, GC-MS analysis can be more accurate, and provide more information on a sample than GC-ECD analysis.

CHAPTER 2: MATERIALS AND METHODS

2.1: FISH OIL SAMPLES:

For this work, eight different fish oil and fish liver oil products were purchased in total. All of the fish oil samples were purchased in capsule form. Three different brands of fish oil capsules were investigated: Boots "EPA" natural fish oil concentrate capsules (The Boots Company PLC, Nottingham, G.B.), Sanatogen "Omega 3" selected marine fish oil capsules (Fisons Consumer Health, Loughborough, G.B.), and Seven Seas "Pulse" selected fish oil capsules (Seven Seas Healthcare Ltd., Hull, G.B.). Five different brands of fish liver oils were purchased, four in capsule form and one as a bottled liquid. The five brands consisted of three cod liver oil products and two halibut liver oil products. The five fish liver oil brands were Sanatogen Cod Liver Oil (CLO) capsules (Fisons Consumer Health, Loughborough, G.B.), Seven Seas Cod Liver Oil capsules (Seven Seas Healthcare Ltd., Hull, G.B.), Crookes Halibut Liver Oil (HLO) capsules (Crookes Healthcare Ltd., Nottingham, G.B.), Boots Halibut Liver Oil capsules (The Boots Company PLC, Nottingham, G.B.), and Boots Cod Liver Oil B.P. liquid (The Boots Company PLC, Nottingham, G.B.).

2.1.1: EXPERIMENTAL:

The analytical procedures used for the fish liver oil and fish oil products were identical. HPLC was chosen as the method for separating the PCBs from the oil samples, ready for analysis by GC-MS. Previous work (56) led to a 300 x 7.5 mm i.d. Polymer PLRP-S HPLC column (Polymer Labs., Church Stretton, G.B.) being used for this work. The HPLC system used consisted of an LDC Constametric III high pressure pump (LDC (UK) Ltd., Stone, G.B.) and an LDC/Milton Roy Spectromonitor III variable wavelength UV detector (LDC (UK) Ltd., Stone, G.B.). The UV detector was set at a wavelength of 254 nm. All of the solvents used were HPLC grade, and were purchased from FSA Lab. Supplies (Loughborough, G.B.).

A mobile phase of 80 % heptane and 20 % propan-2-ol was used initially at a flowrate of 2.3 ml/min. A sample of oil was carefully removed from the capsule, or bottle, using a graduated syringe. The initial investigations into the behaviour of fish oils on the PLRP-S HPLC column were done using Crookes HLO capsules as an example. 0.5 ml of the Crookes Halibut Liver Oil was removed from a capsule, mixed with 0.5 ml of 100 μg/ml Aroclor 1242, and then injected onto the HPLC column. The fat and solvent came off the column almost immediately, between 4 and 7 minutes after injection. The PCBs eluted more than 8 minutes after injection, as two peaks with the second peak being very broad. The identity of the PCB peaks was confirmed by capillary column GC-MS. Although the PCBs were separated from the oil, the fact that the PCBs came off within a couple of minutes of the fat was not welcome. Therefore, a different mobile phase was required, and 60 % heptane and 40 % propan-2-ol was chosen to attempt to increase the retention of the PCBs. This new mobile phase was used with the same mixture of oil and PCBs as tried with the previous mobile phase.

The oil and the solvent again eluted between 4 and 7 minutes after injection. The Aroclor 1242 eluted as a single peak over approximately 5 minutes, with the peak maximum at around 10 minutes after injection. A sample of 28 μg/ml Aroclor 1260 was also injected with an aliquot of the oil, in the same way as the Aroclor 1242, to give an idea of the behaviour of the full range of PCB congeners under this set of conditions. The Aroclor 1260 was found to elute over much the same time window as the Aroclor 1242, with the peak maximum slightly later than the Aroclor 1242, at 10.5 minutes after injection. A sample of ^{13}C-labelled PCBs, which would be used as recovery standards with the real samples (see Section 2.1.2), was also tested under the same conditions. The ^{13}C-labelled PCBs were observed as three peaks at 8.8 minutes, 10.4 minutes, and 13.8 minutes after injection.

These initial results were promising, and so a mobile phase of 60 % heptane and 40 % propan-2-ol was adopted for the analysis of the eight fish oil and fish liver oil samples. The eluents from the HPLC column were collected in test tubes between 7.5 and 17 minutes, and analysed for

PCBs by capillary column GC-MS. The extraction of each individual fish oil sample proceeded as follows:

Four 75 µl aliquots of each different fish oil sample were removed with the syringe, and 50 µl of the ^{13}C-labelled PCB recovery surrogates were added to each aliquot, along with 1 ml of the HPLC mobile phase (60 % heptane and 40 % propan-2-ol). The four aliquots of each fish oil sample were injected separately onto the HPLC column, and all of the eluents were collected in test tubes between 7.5 minutes and 17 minutes after injection. The volumes of the eluents were reduced under a stream of nitrogen, and the four eluents of each fish oil sample were bulked together.

The final volume of this bulked sample was reduced to less than 1.5 ml, and the sample injected onto the HPLC column for a second time. This second HPLC step was necessary to ensure that none of the original oil found its way into the final extract, because the oil would quickly cause a loss of vital sensitivity of the GC-MS. The eluent was collected as before from the HPLC between 7.5 minutes and 17 minutes after injection. The final volume of this eluent was reduced to less than 1 ml, and 100 µl of the internal standard, 4,4'-dibromobiphenyl (Aldrich Chem. Co. Ltd., Gillingham, G.B.), was added. The final volume of the extract was adjusted to exactly 1 ml with heptane, using a 5 decimal place digital balance.

A column cleaning step was performed between each HPLC injection. A mobile phase of 100 % heptane was pumped through the column for 15 minutes, to ensure that all of the lipid material from the fish oils was removed from the column.

All of the samples were analysed on a Hewlett-Packard HP 5890 gas chromatograph with a 5970 MSD mass spectrometer and a 7673A automatic sampler (Hewlett-Packard, Bracknell, G.B.). A 50 m x 0.2 mm i.d. HP-1 capillary column (Hewlett-Packard, Bracknell, G.B.), with a film thickness of 0.33 µm, was used for the analyses. Hydrogen was used as the GC carrier gas, with a volumetric flow rate set at 1 ml/min. Splitless injection was used, and 5 µl of sample was injected throughout. A selected ion monitoring (SIM) programme specifically designed for the analysis of PCBs was used. The temperature programme consisted of an initial temperature of 75

^{o}C held for 2 minutes. This was followed by two temperature ramps, firstly 30 ^{o}C/min to 120 ^{o}C, then 10 ^{o}C/min to 240 ^{o}C. This final temperature of 240 ^{o}C was held for 20 minutes.

The SIM programme used meant that at a given time the mass spectrometer was searching for 4 specific mass values. The 4 mass values represented two masses for each of two levels of chlorination, e.g. monochloro and dichlorobiphenyls. All of the samples were analysed in duplicate, and the average results were reported. The actual SIM ions used are shown in Table 2.1.

LEVEL OF CHLORINATION	m/z VALUES	
	PRIMARY	SECONDARY
1Cl	188.1	190.0
2Cl	222.0	224.0
3Cl	256.0	258.0
4Cl	291.9	289.8
5Cl	325.8	327.8
6Cl	359.7	361.7
7Cl	393.8	395.8
8Cl	429.8	431.9
9Cl	463.8	465.8
10Cl	497.8	499.8
$C_{12}H_8Br_2$ (I.S.)	311.9	313.9

TABLE 2.1: ACTUAL IONS RECORDED IN A SIM ANALYSIS

2.1.2: QUANTITATION OF PCBS:

The quantitation of the PCBs present was achieved by using an external calibration standard called RPCBR-1 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), which contained two mono-substituted PCB congeners and one congener for each of the other nine chlorination levels. The standard solution supplied by Greyhound contained eleven individual PCB congeners, each at a concentration of 1000 μg/ml. The eleven PCBs present were No.s 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, 209 (5). Each PCB congener selected by the company had a GC

response factor which was representative of the response factors of the other PCB congeners of that chlorination level.

A set of solutions of this RPCBR-1 standard at different concentrations was made up and run on the GC-MS, using the SIM programme specially developed for PCBs. The original solution, containing the PCB congeners at a concentration of 1000 μg/ml, was diluted 1 in 100, in two steps of 1 in 10 dilution, with HPLC grade heptane (FSA Lab. Supplies, Loughborough, G.B.), to give a 10 μg/ml solution of the congeners. A 500 ng/ml solution of the congeners was also prepared. These solutions were used as the stock solutions from which a set of calibration solutions were prepared. Six calibration solutions were prepared, containing the PCB congeners at concentrations of 5 μg/ml, 1 μg/ml, 500 ng/ml, 250 ng/ml, 100 ng/ml, and 50 ng/ml, respectively. All of the standards had 4,4'-dibromobiphenyl present as an internal standard at a concentration of 1 μg/ml (or 1 ppm), exactly as in the fish oil extracts. Each of these standards was prepared in a clean 10 ml volumetric flask. Firstly, the required volume of the RPCBR-1 stock solution was added to the flask using a graduated syringe, then 1 ml of a 10 μg/ml solution of 4,4'-dibromobiphenyl was added using another graduated syringe. Finally, the volume was made up to exactly 10 ml with HPLC grade heptane using a pasteur pipette. The 10 μg/ml stock solution was used to prepare the 5 μg/ml, 1 μg/ml, and 500 ng/ml standards, while the 500 ng/ml stock solution was used to prepare the 250 ng/ml, 100 ng/ml, and 50 ng/ml standards. The six standard solutions were run on the GC-MS.

Calibration curves for each of the ten chlorination levels were constructed, with the peak area of the PCB congener of interest divided by the peak area of the internal standard (4,4'-dibromobiphenyl) and plotted against the known concentrations of the RPCBR-1 solutions. Each calibration curve was plotted on a computer, and a statistical package called INSTAT, developed by Dr. P. Christie of Bath University Computing Services (BUCS), was used. This statistical package was available on the computer network in the School of Pharmacy & Pharmacology. The statistical package calculated the calibration curve, the line of regression of y on x, by the least

squares method, and reported the correlation coefficient, r. The computer also reported the slope (m) and intercept (c) of the calibration curve, from the general equation for a straight line,

$y = mx + c$.

These ten calibration curves were then used to calculate the amounts of PCBs in the fish oil samples by chlorination level (homologue). The use of an internal standard allowed for any variation in the actual volume of sample injected by the GC autosampler. Each fish oil extract was analysed in duplicate, results were calculated by homologue, and the average results have been reported.

The SIM programme used for these analyses ensured that each peak recorded by the GC-MS had to pass a test before being accepted as a PCB peak. At each level of chlorination, peak areas at two different masses were recorded. The ratio of the two peak areas had to fall within pre-set limits (Table 2.2), which were different for each level of chlorination, before any peak was accepted as a PCB peak. If the experimental peak area ratio was greater than +/-20 % of the theoretical value, the peak was rejected from the quantitation procedure. Only the peak areas from one mass value, the primary ion, were used to quantify the PCBs at each level of chlorination.

HOMOLOGUE	ION (RELATIVE INTENSITY)		
	PRIMARY	SECONDARY	TERTIARY
$C_{12}H_9Cl$	188 (100)	190 (33)	-
$C_{12}H_8Cl_2$	222 (100)	224 (66)	226 (11)
$C_{12}H_7Cl_3$	256 (100)	258 (99)	260 (33)
$C_{12}H_6Cl_4$	292 (100)	290 (76)	294 (49)
$C_{12}H_5Cl_5$	326 (100)	328 (66)	324 (61)
$C_{12}H_4Cl_6$	360 (100)	362 (82)	364 (36)
$C_{12}H_3Cl_7$	394 (100)	396 (98)	398 (54)
$C_{12}H_2Cl_8$	430 (100)	432 (66)	428 (87)
$C_{12}HCl_9$	464 (100)	466 (76)	462 (76)
$C_{12}Cl_{10}$	498 (100)	500 (87)	496 (68)
$C_{12}H_8Br_2$[a]	312 (100)	314 (50)	-
$^{13}C_6{}^{12}C_6H_9Cl$[b]	194 (100)	196 (33)	-
$^{13}C_{12}H_6Cl_4$[b]	304 (100)	306 (49)	302 (76)
$^{13}C_{12}H_2Cl_8$[b]	442 (100)	444 (65)	440 (87)
$^{13}C_{12}Cl_{10}$[b]	510 (100)	512 (87)	508 (68)

a - Internal standards added to sample before GC-MS analysis
b - Recovery surrogates added to sample before extraction

TABLE 2.2: CHARACTERISTIC SIM ION RATIOS FOR PCBS

From Erickson (1), pg. 219

The fish oil extracts were also run on the GC-MS to calculate the experimental percentage recoveries using a separate SIM programme specifically designed for the analysis of the ^{13}C-labelled PCBs. The ^{13}C-labelled PCB standard, called C-063, was purchased from the United States Environmental Protection Agency (Research Triangle Park, U.S.A.), specifically for use as a PCB recovery surrogate. The original solution, C-063, was diluted 100-fold to yield a solution, called SS001, ready for use. The SS001 solution contained four ^{13}C-labelled PCB congeners, (1', 2', 3', 4', 5', 6', -$^{13}C_6$) 4-chlorobiphenyl at 1.0 μg/ml, ($^{13}C_{12}$) 3, 3', 4, 4'-tetrachlorobiphenyl at 2.5 μg/ml, ($^{13}C_{12}$) 2, 2', 3, 3', 5, 5', 6, 6'-octachlorobiphenyl at 4.0 μg/ml, and ($^{13}C_{12}$) decachlorobiphenyl at 5.0 μg/ml. These four congeners have been assigned the congener numbers 211, 212, 213, 214 by the manufacturers, and are the ^{13}C-labelled equivalents of the PCB congeners No. 3, 77, 202, and 209. The solution was designed so that when diluted by 1 in 10 in a final extract, the concentrations of the four ^{13}C-labelled PCB surrogates would fall within the operating range of most mass spectrometers.

Calibration curves for each of the four ^{13}C-labelled PCBs were constructed by running 4 solutions of SS001 at different concentrations, with 4,4'-dibromobiphenyl present in all the solutions at a concentration of 1 μg/ml. The 4 solutions were made by diluting the SS001 standard by 1 in 5, 1 in 10, 2 in 25, and 1 in 20 using the appropriate volumetric flasks.

The peak areas of the four ^{13}C-labelled PCBs in the recovery standard were divided by the peak area of the internal standard, and calibration curves for each congener were drawn by plotting this ratio against the known concentrations of the SS001 solutions on a computer. The statistical package INSTAT, which plotted a straight line through the points by the method of least squares, was used for this. The calculated slopes (m), intercepts (c), and correlation coefficients (r) were recorded. The experimental percentage recovery of each individual fish oil sample could then be calculated.

Each final fish oil extract had 200 μl of the SS001 standard added to it originally. The final volume of each extract was 1 ml, and therefore the extracts should contain a 1 in 5 dilution of the SS001 standard assuming a 100 % experimental recovery. The actual experimental percentage

recoveries were calculated by comparison of the experimental peak height ratios of the ^{13}C-labelled PCBs with their calibration curves. The ^{13}C-labelled PCB congener No.212, ($^{13}C_{12}$) 3, 3', 4, 4'-tetrachlorobiphenyl, was used to calculate the experimental percentage recoveries of the extracts. This was because, as a tetrachlorobiphenyl, its behaviour most closely matched that of the majority of PCB congeners found in the fish oil extracts.

2.2: EGG SAMPLES:

2.2.1: EXPERIMENTAL:

Seven composite duck egg samples were analysed. The eggs had been collected from a site near an industrial incineration plant at Pontypool in Wales. The eggs in the seven samples had been removed from their shells and mixed thoroughly. The composite samples were stored in a freezer until they were analysed. All of the samples were yellow in colour. Each sample was identified by a different two letter code. The seven egg samples were called KD, LF, LN, LO, LR, LS, and LT.

Approximately 10 g of each egg sample was accurately weighed on a digital balance. Each egg sample was mixed with 30 g of anhydrous sodium sulphate (Aldrich Chem. Co. Ltd., Gillingham, G.B.) (73) using a glass mortar and pestle, and the mixture was then allowed to dry. Once the mixture had dried, it was powdered and transferred to an 80 mm x 28 mm i.d. Soxhlet extraction thimble (Whatman Scientific Ltd., Maidstone, G.B.). 100 µl of the ^{13}C-labelled PCB recovery standard, SS001 (United States Environmental Protection Agency, Research Triangle Park, U.S.A.), was added to each egg sample with an automatic pipette. The egg sample was then extracted overnight in a Soxhlet extraction apparatus with approximately 150 ml of HPLC grade hexane (FSA Lab. Supplies, Loughborough, G.B.).

The volume of the hexane extract was reduced to approximately 5 ml, in 30 minutes, using a Kuderna-Danish apparatus, made to a design supplied by the Laboratory of the Government Chemist. This was done as follows: The extract was transferred, with careful hexane washing, to

a graduated quickfit 10 ml centrifuge tube. The centrifuge tube was connected to a quickfit 250 ml extraction bulb (a Kuderna-Danish flask). A quickfit side-arm with a splash-bulb was attached to the top of the extraction bulb, and the side-arm was connected to a quickfit condenser. The end of the condenser was placed in the top of a 100 ml measuring cylinder. The centrifuge tube and extraction bulb were positioned inside a steam cabinet, which was thermostatically controlled at approximately 80 ^{o}C. As the tube and bulb heated up, the hexane boiled over through the condenser, and collected in the measuring cylinder. The PCB-containing fraction, however, remained in the centrifuge tube. When the volume of the PCB-containing fraction had been reduced to approximately 5 ml, the tube was removed from the steam cabinet. The volume of the extract was then carefully reduced to a final volume of less than 3 ml using a nitrogen line, with a steady flow of nitrogen and gentle warming.

The extract now contained all of the PCBs and lipids present in the original egg sample. The next step was to separate the PCBs from the lipids. This was achieved by using a 300 x 7.5 mm i.d. Polymer PLRP-S HPLC column (Polymer Labs., Church Stretton, G.B.). The HPLC system used consisted of an LDC Constametric III high pressure pump (LDC (UK) Ltd., Stone, G.B.) and an LDC/Milton Roy Spectromonitor III variable wavelength UV detector (LDC (UK) Ltd., Stone, G.B.). A mobile phase of 65 % heptane and 35 % propan-2-ol was used at a flow rate of 2.3 ml/min. This particular experimental mobile phase was used as a result of further work following on from the work reported in Section 2.1.1. A Rheodyne injection valve, with a 2.0 ml injection loop, was used to introduce the sample onto the column.

The lipids eluted less than 7 minutes after injection, and the PCB fraction was collected in clean test tubes between 7 and 17 minutes after an injection. After 17 minutes, the mobile phase was pumped through the column for a further 10 minutes before the next injection was made. Each of the egg extracts was injected onto the column as two separate 1.5 ml aliquots. Once both aliquots had been injected, and the two PCB fractions had been collected, the fractions were combined, and the final volume reduced from approximately 50 ml to approximately 1.5 ml using a nitrogen line, with a steady stream of nitrogen and gentle warming.

The whole extract was then injected onto the HPLC column for a second time, and the PCB fraction was again collected. The volume of this final extract was again reduced, this time to less than 10 ml. This second HPLC step was done to reduce the amount of lipid present in the final extract.

Once the PCB fractions from a single egg extract had been eluted from the HPLC column, the column was washed for fifteen minutes, with pure heptane as the mobile phase, at a flow rate of 1.5 ml/min. Only after this step were the aliquots of the next egg extract injected onto the HPLC column. This was to ensure that no lipids, PCBs or other compounds from the previous egg extract were carried over into the next egg extract, producing erroneous results.

The egg extracts prior to the HPLC step were a strong yellow colour. This yellow colour eluted from the HPLC column mostly in the lipid fraction, but the PCB fractions eluting from the HPLC column were also slightly yellow in colour. Even after the second HPLC step, the PCB extracts still retained a slight yellow colouration. Therefore, a step was used to eliminate the yellow colour from the extracts before analysis by GC, to ensure that the performance of the GC was not detrimentally affected by substances other than those of interest being present in the final extracts.

The extracts were treated with concentrated sulphuric acid to remove the yellow colour that was present (74). 20 ml of concentrated sulphuric acid was added to each extract, which was less than 10 ml in volume, in a 50 ml separating funnel. The funnel was shaken vigorously, and the pressure build-up in the funnel was carefully let out by opening the valve of the funnel, while it was inverted. The separating funnel was then allowed to stand in a fume cupboard for 1 hour. The lower acid layer was discarded, and the now colourless heptane layer was carefully washed with two small volumes of distilled water, to remove any traces of the acid that remained. A little magnesium sulphate was added to the wet heptane extract in the separating funnel, and the extract was allowed to stand for several minutes. The extract was then filtered, with heptane washing, through Whatman 1PS phase separating paper (Whatman Scientific Ltd., Maidstone, G.B.) into a clean, dry heptane-washed test tube.

After the sulphuric acid step was completed, the volumes of the final extracts were reduced to less than 1 ml with a nitrogen line. 100 µl of an approximately 5 ppm solution of PCBs No. 30 and 209 was added to each of the extracts. Both PCB congeners were used to calculate the experimental GC relative retention times, while PCB No. 209 was used as the internal standard for quantitation. The final volume of each extract was then carefully adjusted to exactly 1 ml using heptane. This volume was checked, and corrected using a 5 decimal place digital balance.

All of the individual egg extracts were analysed by an 8320B capillary column GC-ECD (Perkin-Elmer, Beaconsfield, G.B.) with a ^{63}Ni electron capture detector. A 50 m x 0.2 mm i.d. HP-1 column (Hewlett-Packard, Bracknell, G.B.), with a film thickness of 0.33 µm, was used for the analyses. The splitless injector temperature was set at 250 °C, and the detector temperature at 290 °C. Hydrogen was used as the carrier gas, at a pressure of 6 p.s.i., with nitrogen as the make-up gas. A temperature programme was developed for the analyses. This consisted of an initial temperature of 50 °C for 1 minute, followed by temperature ramps of 30 °C/min to 150 °C and then, 2.5 °C/min to 270 °C. The final temperature of 270 °C was held for 10 minutes. All of the chromatograms were plotted out on a GP-100 graphics plotter (Perkin-Elmer, Beaconsfield, G.B.), and peak heights were measured manually.

An extraction of just anhydrous sodium sulphate was done to provide a blank for comparison with the actual egg extracts. The anhydrous sodium sulphate was extracted and cleaned up in exactly the same way as the egg extracts. The same amount of PCBs No. 30 and 209 were added to the blank extract, and it was run by capillary column GC-ECD using the same temperature programme as for the egg extracts. An injection of pure heptane was also made on the GC-ECD using the same temperature programme, to find out if the solvent gave peaks which could interfere with the egg extract analyses.

2.2.2: QUANTITATION OF PCBS BY GC-ECD:

A standard solution, called PCB Mix No.3, containing the individual PCB congeners No. 28, 52, 101, 118, 138, 153, and 180 was purchased (British Greyhound Chromatography & Allied

Chemicals, Birkenhead, G.B.). The standard solution was a 10 ml solution of the 7 PCB congeners, each at a concentration of 10 μg/ml, in iso-octane. This solution was diluted 1 in 10 with heptane in a 10 ml volumetric flask, to give a solution containing the 7 PCB congeners each at a concentration of 1 μg/ml, or 1 ppm. The 10 μg/ml solution was diluted 1 in 100 in exactly the same way to give a solution containing the 7 PCB congeners each at a concentration of 100 ng/ml, or 100 ppb. After analysing both diluted solutions, it was found that the 100 ppb solution gave a reasonable response on the GC-ECD, and it was decided to use this as the stock solution to prepare future standard solutions.

The 7 individual PCB congeners used in this work are used by an international standards committee (ICES) to compare the PCB content of various samples. The method is referred to in this thesis as the ICES Method. The congeners were used in Dutch legislation, where all regulations on PCB content concern maximum permitted levels of any one of the 7 congeners. A similar method is in place in Germany, where 6 of the 7 PCB congeners are used in the same way. The 7 congeners were those found to be most prevalent in a range of sample matrices.

The PCB congeners 30 and 209 were obtained as individual solids (Ultrascientific Inc., Hope, U.S.A.). A small amount of each congener was accurately weighed out and dissolved in heptane. 0.00206 g of PCB No. 30 was weighed out and dissolved in 10 ml of heptane in a volumetric flask, and 0.00176 g of PCB No. 209 was weighed out and dissolved in 10 ml of heptane in a volumetric flask. 1 ml of each solution was pipetted into a 10 ml volumetric flask, and heptane was added to give a final volume of 10 ml. This solution contained PCB No. 30 at a concentration of 20.6 μg/ml, and PCB No. 209 at a concentration of 17.6 μg/ml. 5 ml of this solution was pipetted into a 20 ml volumetric flask and the volume made up with heptane. This solution then contained PCB No. 30 at a concentration of 5.15 μg/ml, and PCB No. 209 at a concentration of 4.40 μg/ml.

A set of calibration solutions were made by taking a series of different volumes of the 100 ng/ml solution of 7 PCB congeners (900 μl, 800 μl, 600 μl, 500 μl, 350 μl, 100 μl, 50 μl) using a graduated syringe, and adding 100 μl of the solution containing 5.15 μg/ml PCB No. 30 and 4.40

μg/ml PCB No. 209 to each, using another graduated syringe. Each solution was made up to a total volume of exactly 1 ml with heptane. This approach gave seven standard solutions with concentrations of the 7 PCB congeners of 90 ng/ml, 80 ng/ml, 60 ng/ml, 50 ng/ml, 35 ng/ml, 10 ng/ml, and 5 ng/ml, with PCB No.30 at 0.515 μg/ml and PCB No.209 at 0.44 μg/ml present in each sample. Every egg sample that was analysed had 100 μl of the same solution of PCBs No.30 and 209 added to it with the same graduated syringe, to allow direct comparison of the samples with the standards.

Each of the standards was run in duplicate on the capillary GC-ECD. Identification of PCBs in the egg samples was done by comparison of the experimental relative retention times of the peaks in the samples with those of the standards. The experimental relative retention times (ERRT) of the 7 congeners in the standards were calculated using equation (1):

$$ERRT_x = \frac{t_x - t_{30}}{t_{209} - t_{30}} \qquad -(1)$$

where, x = chosen peak

30 = congener 30

209 = congener 209

t = retention time in mins

The ERRTs for the samples were calculated using the same formula. The sample ERRT values were then compared with the ERRT values of the standards. A sample peak was said to be due to one of the 7 congeners of interest if the ERRT of the sample peak equalled the ERRT of the standard peak to within +/- 0.1.

Once the PCB congeners had been identified, the next step was to plot calibration curves for each congener. The peak heights of each of the 9 PCB congeners present were measured manually from the chromatograms printed out by the plotter. The peak heights of each of the 7 congeners in PCB Mix No. 3 were divided by the peak height of PCB No. 209. For each of the 7 congeners,

this ratio was plotted against the known concentration of the congener in the seven standard solutions to give a calibration curve for that individual congener.

Each calibration curve was plotted on a computer. A statistical package called INSTAT was used, which calculated the calibration curve, the line of regression of y on x, by the least squares method, and reported the correlation coefficient, r. The computer also reported the slope (m) and intercept (c) of the calibration curve, from the general equation for a straight line,

$y = mx + c$.

The reported values for the slope (m) and intercept (c) for each PCB congener were used to calculate the concentration of the PCB congener (x) present in each egg extract, once the experimental peak height ratio (y) had been calculated, by using the equation, $y = mx + c$. All of the egg extracts were analysed in duplicate, and the average results for each of the 7 individual congeners were recorded.

The concentration of each of the 7 individual PCB congeners in the egg samples was calculated. These results were then divided by the weight of each egg sample that had been extracted. This gave a concentration for each of the 7 congeners per gram of egg. A Total PCB concentration was calculated by adding together the concentrations of the 7 individual congeners, and multiplying the sum by a factor of four, as specified by the ICES Method.

2.3: COWS MILK SAMPLES:

2.3.1: EXPERIMENTAL:

2.3.1.1: UEA Cows Milk Samples:

Eight cows milk samples were sent from the University of East Anglia (UEA). Each of the eight milk samples was labelled with a two letter code. The eight samples were labelled RA, RD, RG, RJ, RK, RL, RM, and RN. The milk samples were collected and immediately frozen. As soon as the samples arrived in the laboratory, they were freeze-dried. 100 ml of each sample was

measured out into a 250 ml quickfit round bottomed flask using a measuring cylinder. The milk in the flasks was, then, freeze-dried overnight. The freeze-dried milk was transferred to glass sample bottles, and the total weight of each sample of freeze-dried milk was recorded. The sample bottles were stored in the freezer, until the milk samples were required for extraction.

For an extraction, a weighed amount of freeze-dried milk, equivalent to 10 ml of fresh milk, was placed in a glass mortar. An amount of 60-100 mesh Reagent Grade Florisil (Aldrich Chem. Co. Ltd., Gillingham, G.B.), enough to two-thirds fill a Soxhlet extraction thimble, was added to the mortar. The mixture was ground to a fine running powder with a glass pestle. The mixture was packed into an 80 mm x 28 mm i.d. Soxhlet extraction thimble (Whatman Scientific Ltd., Maidstone, G.B.), 100 µl of the ^{13}C-labelled PCB standard SS001 (United States Environmental Protection Agency, Research Triangle Park, U.S.A.) was added with a syringe, and the mixture was extracted overnight with 150 ml of HPLC grade hexane (FSA Lab. Supplies, Loughborough, G.B.), using a Soxhlet apparatus.

The volume of the hexane extracts was reduced in exactly the same way as reported in Section 2.2.1 using a Kuderna-Danish apparatus. The volume of the extracts was reduced to about 10 ml, and then a nitrogen line was used to reduce the volume to less than 3 ml. The same HPLC method was used to separate the PCBs from the lipids in each extract as in Section 2.2.1. The original extracts were injected onto the HPLC column as two separate aliquots. The PCB fractions collected were combined, and the total volume reduced to less than 1.5 ml using a nitrogen line. This solution was injected onto the HPLC column, and the PCB fraction, again, collected.

The volume of this final extract was reduced to less than 1 ml using a nitrogen line. 100 µl of the approximately 5 ppm solution of PCBs No. 30 and 209 was added to each solution, and the final volume was made up to 1 ml with HPLC grade heptane, using a 5 decimal place digital balance, just as for the egg samples.

All of the cows milk samples were analysed in duplicate by capillary column GC-MS. An HP5890 gas chromatograph with a 5970 MSD mass spectrometer and a 7673A automatic sampler was used (Hewlett-Packard, Bracknell, G.B.). A 50 m x 0.22 mm i.d. BPX-5 column, with a film

thickness of 0.25 μm, was used for the analyses. Hydrogen was the GC carrier gas, with a volumetric flow rate of 1 ml/min. Cold on-column injection was used, and 2 μl of each extract was injected by a syringe with a fused silica needle. A temperature programme was used for the analyses of the cows milk extracts. This consisted of an initial temperature of 50 °C held for 1 minute, followed by a temperature ramp of 40 °C/min to 130 °C. Four further temperature ramps followed, of 2.5 °C/min to 170 °C, then 0.5 °C/min to 190 °C, 1.0 °C/min to 210 °C, and 1.5 °C/min to 290 °C. The final temperature of 290 °C was held for 5 minutes.

The SIM programme that was used meant that at any given time during a GC analysis the mass spectrometer was searching for 4 specific mass values. The 4 mass values represented two masses for each of two levels of chlorination, Section 2.1.2. All of the cows milk extracts were analysed in duplicate, and the average results were reported.

2.3.1.2: MAFF Cows Milk Samples:

For this work, the cows milk samples were provided by the Ministry of Agriculture, Fisheries and Food (MAFF) from the milk stocks of two different dairy farms in Cheshire, England. The samples were labelled "J & G Jackson, Teuthill Farm, Alvanley" and "J. B. Allwood & Sons, Milton Brook Lodge, Great Barrow, Cheshire". Cows milk samples were collected from the two farms by MAFF on a weekly basis over a period of 12 weeks. Information was supplied by MAFF that Teuthill Farm was situated close to a disused landfill site, while Milton Brook Lodge was about five miles further away from the landfill site. The Ministry was concerned that the Teuthill Farm milk may have become contaminated with PCBs, which had been dumped at the nearby landfill site.

The milk samples were sent by courier in screw-topped glass bottles, two bottles of each sample. Upon arrival, 50.0 ml of milk was accurately measured out from each bottle using measuring cylinders. Each 50.0 ml sample of milk was transferred to a 250 ml round-bottomed flask, and freeze-dried overnight. The freeze-dried milk samples were carefully transferred to screw-capped glass bottles, weighed, and then stored in a freezer until required.

The extraction procedure began with an amount of freeze-dried milk being weighed out, which corresponded to 10 ml of the original cows milk sample. The freeze-dried milk was then mixed with enough of a 50:50 Florisil:cellulose mixture to fill a Soxhlet thimble. The 60-100 mesh Florisil was purchased from Aldrich Chem. Co. Ltd. (Gillingham, G.B.), and the fibrous cellulose powder was purchased from Whatman Scientific Ltd. (Maidstone, G.B.). The milk and Florisil:cellulose were mixed with a glass mortar and pestle. The mixture was then transferred to an 80 mm x 28 mm i.d. Soxhlet thimble (Whatman Scientific Ltd., Maidstone, G.B.). 100 µl of the ^{13}C-labelled PCB recovery standard SS001 (United States Environmental Protection Agency, Research Triangle Park, U.S.A.) was added to the thimble, and the mixture was extracted overnight with about 150 ml of HPLC grade hexane (FSA Lab. Supplies, Loughborough, G.B.).

The volume of the resultant hexane extract was then reduced using a Kuderna-Danish apparatus, exactly as described in Section 2.2.1. The total volume of the extract was reduced to about 5 ml. The volume was then carefully reduced to a final volume of about 3 ml using a nitrogen line, with a steady stream of nitrogen and gentle warming.

The 3 ml extract contained the organic compounds of interest as well as all of the fat from the cows milk. The next step was to separate the fat from the organic compounds. This was achieved using the semi-preparative HPLC method, which was detailed in Section 2.1.1. A 300 x 7.5 mm i.d. Polymer PLRP-S column (Polymer Labs., Church Stretton, G.B.) was again used. The mobile phase that was used consisted of 65 % heptane and 35 % propan-2-ol, at a flow rate of 2.3 ml/min. A total analysis time of less than 30 minutes was required.

Each extract was injected onto the HPLC column as two separate 1.5 ml aliquots, using a 2 ml injection loop. The lipids eluted less than 7 minutes after injection, and the PCB fraction was collected in clean test tubes between 7 and 17 minutes after an injection. After 17 minutes, the mobile phase was pumped through the column for a further 10 minutes before the next injection was made. Once both aliquots had been injected, and the two PCB fractions had been collected, the fractions were combined, and the final volume reduced from approximately 50 ml to 1.5 ml using the nitrogen line, with a steady stream of nitrogen and gentle warming.

The whole extract was then injected onto the HPLC column, and the PCB fraction was again collected. The volume of this final extract was again reduced, this time to less than 1 ml. This second HPLC step was done to reduce the amount of lipid present in the final extract. 100 µl of the internal standard, 4,4'-dibromobiphenyl (Aldrich Chem Co. Ltd., Gillingham, G.B.), was added to the extract with a graduated syringe. The final volume of the extract was then carefully adjusted to exactly 1 ml using a syringe. This meant that each extract contained the internal standard at a concentration of 1 µg/ml.

All of the samples were analysed on a Hewlett-Packard HP 5890 gas chromatograph with a 5970 MSD mass spectrometer and a 7673A automatic sampler (Hewlett-Packard, Bracknell, G.B.). A 25 m x 0.2 mm i.d. HP-1 capillary column (Hewlett-Packard, Bracknell, G.B.), with a film thickness of 0.33 µm, was used for the analyses. Hydrogen was used as the GC carrier gas, with a volumetric flow rate set at 1 ml/min. Splitless injection was used, and 5 µl of sample was injected throughout. A selected ion monitoring (SIM) programme specifically designed for the analysis of PCBs was used. The temperature programme consisted of an initial temperature of 75 ^{o}C held for 2 minutes. This was followed by two temperature ramps, firstly 30 ^{o}C/min to 120 ^{o}C, and then 10 ^{o}C/min to 240 ^{o}C. The final temperature of 240 ^{o}C was held for 20 minutes.

The SIM programme used meant that at a given time the mass spectrometer was searching for 4 specific mass values. The 4 mass values represented two masses for each of two levels of chlorination. All of the samples were analysed in duplicate, and the average results were reported.

2.3.2: QUANTITATION OF PCBS:

2.3.2.1: UEA Cows Milk Samples:

Eight cows milk samples were analysed, called RA, RD, RG, RJ, RK, RL, RM, and RN. The eight samples were analysed in two groups of four, with a blank extract in each batch. The blanks consisted of pure Florisil, which was treated in exactly the same way as the cows milk samples.

The results of the eight cows milk samples were quantified, using the 7 component PCB Mix No. 3 as the quantitation standard. The PCB Mix No. 3 contained the 7 individual PCB congeners PCB No. 28, 52, 101, 118, 138, 153, and 180. Exactly the same standard solutions as in Section 2.1.2 were used for this quantitation work.

The standard solutions of the 7 individual PCB congeners were used to calculate the experimental relative retention times (ERRTs) for each congener. Exactly the same equation as in Section 2.1.2 was used to calculate the ERRTs:

$$ERRT_x = \frac{t_x - t_{30}}{t_{209} - t_{30}} \quad - (1)$$

where, x = chosen peak

30 = PCB congener No. 30

209 = PCB congener No. 209

t = retention time from injection

The retention times were recorded by the GC-MS, and the ERRTs were worked out on a calculator. ERRTs for the peaks found in the cows milk samples were calculated, and a sample peak was accepted as being due to one of the seven PCB congeners of interest only if the sample peak ERRT equalled the target peak ERRT to within +/-0.1.

Once the sample peaks of interest had been identified, and their experimental peak areas recorded, the concentrations of the 7 individual PCB congeners of interest in each cows milk sample were calculated by the ICES Method.

The method involved calculating the relative response factors (RRF) for each of the 7 target PCB congeners using equation (2):

$$RRF = \frac{A_2 \times M_1}{A_1 \times M_2} \qquad \text{- (2)}$$

where, A_2 = peak area of target congener

M_1 = mass of PCB No. 209

A_1 = peak area of PCB No. 209

M_2 = mass of target congener

The RRFs were calculated for each of the 7 individual PCB congeners in each of the PCB Mix No. 3 standard solutions, and the mean RRF values for each congener were used for the quantitation work. The concentrations of each PCB congener in a sample were calculated using equation (3):

$$C_i = \frac{C_s \times A_i \times RRF_s}{A_s \times RRF_i} \qquad \text{- (3)}$$

where, C_i = concentration of congener i

C_s = concentration of PCB No. 209

A_i = peak area of congener i

A_s = peak area of PCB No. 209

RRF_i = RRF of congener i

RRF_s = RRF of PCB No. 209 = 1

The peak areas A_i and A_S were measured and reported by the GC-MS, the concentration of PCB No. 209, C_S, was known, and the RRFs of each congener, RRF_i, were known. Therefore, the concentrations of each of the 7 congeners, C_i, could be calculated using equation (3). A Total PCB concentration was calculated by adding together the concentrations of the 7 individual congeners and multiplying the sum by a factor of four, as specified by the ICES Method. Each of the cows milk samples was analysed in duplicate, and the mean results have been reported in Table 4.1. All of the results have been corrected for the calculated experimental percentage recoveries.

The experimental percentage recoveries were calculated by analysing the ^{13}C-labelled PCBs, which were added to the cows milk samples prior to extraction (Section 2.3.1). The ^{13}C-labelled PCB recovery standards were analysed on the GC-MS separately from the unlabelled PCBs, using a different SIM programme. A set of 4 standard solutions containing the 4 ^{13}C-labelled PCB congeners (SS001) at different known concentrations were made, Section 2.2.2. Each of the 4 solutions also contained the PCBs No. 30 and 209 at exactly the same concentrations as added to the 8 cows milk extracts, to allow direct comparison of standards and samples. The 4 solutions were made by diluting the SS001 standard by 1 in 5, 1 in 10, 2 in 25, and 1 in 20, in volumetric flasks.

Calibration curves for the 4 ^{13}C-labelled PCB congeners were constructed in exactly the same way as in Section 2.2.2. The calibration curves were plotted on a computer using the statistical package called INSTAT. The slopes (m), intercepts (c), and correlation coefficients (r) were recorded.

100 µl of SS001 was initially added to each cows milk sample and the final volume of the cows milk extracts was 1 ml, therefore each final cows milk extract should contain a 1 in 10 dilution of SS001, assuming 100 % recovery. Comparison of the experimental peak height ratios of the ^{13}C-labelled PCBs with the calibration curves of the ^{13}C-labelled PCBs allowed a percentage recovery for each cows milk extract to be calculated. The ^{13}C-labelled PCB congener No.212, ($^{13}C_{12}$) 3, 3', 4, 4'-tetrachlorobiphenyl, was routinely used to calculate the experimental

percentage recoveries, because as a tetrachlorobiphenyl its behaviour most closely matched that of the majority of the 7 individual PCB congeners currently under investigation.

2.3.2.2: MAFF Cows Milk Samples:

The quantitation of the PCBs present was achieved by using an external calibration standard called RPCBR-1 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), which contained two mono-substituted PCB congeners, and one congener for each of the other nine chlorination levels. The standard solution supplied by Greyhound contained the eleven individual PCB congeners at a concentration of 1000 μg/ml each. The eleven PCBs present were No.s 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, and 209. Each PCB congener selected by the company had a GC response factor which was representative of the response factors of the other PCB congeners of that chlorination level.

The same set of 6 standard solutions of the RPCBR-1, with 4,4'-dibromobiphenyl as the internal standard, were used to construct calibration curves as detailed in Section 2.2.2. Calibration curves were constructed for each level of chlorination, and the slopes (m), intercepts (c), and correlation coefficients (r) were recorded. Each calibration curve was plotted on a computer. A statistical package called INSTAT was used which calculated the calibration curve, the line of regression of y on x, by the least squares method. These calibration curves were then used to calculate the concentrations of PCBs in the cows milk extracts, by chlorination level.

The cows milk samples were run in duplicate on the GC-MS. Each peak recorded by the GC-MS had to pass a test before it was accepted as a PCB peak. The peak areas for the two masses monitored for the particular chlorination level in question were ratioed and compared with theoretical values (Table 2.2). The ratio of the two peak areas had to fall within the pre-set limits, which were different for each level of chlorination, before any peak was accepted as a PCB peak. If the experimental peak area ratio was greater than +/-20 % of the theoretical value, the peak was rejected from the quantitation procedure. Only the peak area from one mass value, the primary ion, was used to quantify the PCBs at each level of chlorination.

The same method as detailed in Section 2.2.2 was used to calculate the experimental percentage recoveries of the extracts. The ^{13}C-labelled PCB standard, called C-063, was purchased from the United States Environmental Protection Agency (Research Triangle Park, U.S.A.), specifically for use as a PCB recovery surrogate. The C-063 solution was diluted 100-fold to yield a solution, called SS001, ready for use. Four standard solutions containing the SS001 at different concentrations, as well as the internal standard, were prepared exactly as in Section 2.2.2. Calibration curves for each ^{13}C-labelled PCB congener were constructed, and these were used to calculate the experimental percentage recoveries.

Each final cows milk extract had 100 µl of the SS001 standard added to it originally. The final volume of each extract was 1 ml, and therefore the extracts should contain a 1 in 10 dilution of the SS001 standard, assuming a 100 % experimental recovery. The actual experimental percentage recoveries were calculated by comparison of the experimental peak area ratios of the ^{13}C-labelled PCBs with their calibration curves. The ^{13}C-labelled PCB congener No.212, ($^{13}C_{12}$) 3, 3', 4, 4'-tetrachlorobiphenyl, was used to calculate the experimental percentage recoveries of the extracts. This was because, as a tetrachlorobiphenyl, its behaviour most closely matched that of the majority of PCB congeners found in the cows milk extracts.

There were three important differences between the GC-MS quantitation method used in the work reported here and the use of an Aroclor as an external standard:

(i) Each peak recorded by the GC-MS had to pass a mathematical test before it was accepted as a PCB peak for quantitation. The peak areas for the two masses, monitored for a particular level of chlorination, were ratioed and compared with the theoretical values in Table 2.2. The ratios of the two peak areas had to fall within pre-set limits before any peak was accepted as a PCB peak. If the experimental peak area ratio was greater than +/-20 % of the theoretical value, the peak was rejected from the quantitation procedure. Only the peak areas from one mass value, the primary ion, were used to quantify the PCBs at each level of chlorination.

(ii) PCB congeners can have between 1 and 10 chlorine atoms per molecule. In the GC-MS method used here, the PCBs have been quantified separately by level of chlorination, using a

different PCB congener as a standard for each level. The results which are given as Total PCBs have been calculated by summing the concentrations of all the individual congeners which have been identified. Therefore, the present method allows the composition of PCBs present to be expressed as a total figure, and also by level of chlorination. The use of an Aroclor as a standard means that the composition of PCBs present cannot be expressed by level of chlorination. The current method, therefore, provides additional useful information above that which can be found by the use of an Aroclor for quantitation.

(iii) A known amount of a solution of ^{13}C-labelled PCBs was added to each sample before the extraction commenced. This solution was provided by the United States Environmental Protection Agency, and contained four ^{13}C-labelled PCB congeners with 1, 4, 8, and 10 chlorine atoms per molecule, respectively. After the final extracts had been prepared, a separate GC-MS analysis was carried out to determine the experimental percentage recoveries of the ^{13}C-labelled PCB congeners. These figures were then used as correction factors for the recoveries of the PCB congeners in the extracts. This helped to improve the accuracy of the final PCB concentrations reported.

These three differences helped to ensure that the PCB concentrations reported by the current method would be more accurate than those reported if an Aroclor had been used as the quantitation standard.

2.4: HUMAN BREAST MILK SAMPLES:

2.4.1: EXPERIMENTAL:

A total of 23 breast milk samples were supplied by Bristol University, Department of Child Health. These were collected by midwives from mothers living in the Bristol area on day 7 after childbirth. The samples were identified by a patient number designated by the Department of Child Health. No further details about the individual mothers involved were supplied. The samples were kept in test tubes with plastic caps. These samples were frozen and stored, prior to analysis. The

collection of all of the breast milk samples on day 7 ensured that the results would be directly comparable, and took advantage of the health care visiting arrangements in operation at the time.

For each extraction, the frozen milk was allowed to thaw by leaving the sample in the laboratory. The test tubes were then shaken, and 10 ml of the milk was poured out into a measuring cylinder. This was slowly poured onto 60-100 mesh Florisil (Aldrich Chem. Co. Ltd., Gillingham, G.B.) in a mortar, and mixed with a pestle. The mixture was heated gently, at about 30 ^{o}C, in an oven. Once the mixture had dried, the pestle was used to crush it to a fine running powder. The powder was carefully poured into an 80 mm x 28 mm i.d. Soxhlet thimble (Whatman Scientific Ltd., Maidstone, G.B.), and 100 µl of the ^{13}C-labelled PCB standard SS001 (United States Environmental Protection Agency, Research Triangle Park, U.S.A.) was added with a syringe. The milk was then extracted overnight with 150 ml of HPLC grade hexane (FSA Lab. Supples, Loughborough, G.B.) in a Soxhlet apparatus.

The volume of the extract was initially reduced using a Kuderna-Danish apparatus, described earlier in Section 2.2.1. The volume of the extract was reduced to about 10 ml using the Kuderna-Danish apparatus, and was then further reduced to about 3 ml using a nitrogen line. An HPLC column was used to separate the PCBs in the extracts from the lipids. The HPLC method used for the separation was the same one reported earlier, Section 2.2.1. Each original extract was injected onto the HPLC column as two aliquots of approximately 1.5 ml in volume. The PCB fractions were collected in test tubes, the volumes reduced using a nitrogen line, and the fractions were then combined. The total volume was reduced to about 1.5 ml with a nitrogen line. This solution was then again injected onto the HPLC column, and the final PCB fraction was collected.

The volume of the final extract was reduced to less than 1 ml, and 100 µl of the internal standard, 4, 4'-dibromobiphenyl (Aldrich Chem. Co. Ltd., Gillingham, G.B.), was added with a syringe, Section 2.2.1. The total volume of the extract was adjusted to exactly 1 ml, using a 5 decimal place digital balance.

All of the extracts were analysed in duplicate by capillary column GC-MS. A Hewlett-Packard HP5890 GC with a 5970 MSD mass spectrometer and a 7673A automatic sampler

(Hewlett-Packard, Bracknell, G.B.) was used for the analyses. A 50 m x 0.2 mm i.d. HP-1 capillary column (Hewlett-Packard, Bracknell, G.B.), with a film thickness of 0.33 μm, was used throughout. Hydrogen was the GC carrier gas, at a volumetric flow rate of 1 ml/min. A splitless injection technique was used, and 5 μl of each sample was injected. A selected ion monitoring (SIM) programme was used, with a temperature programme consisting of an initial temperature of 75 °C held for 2 minutes. Two temperature ramps followed, firstly 30 °C/min to 120 °C, then 10 °C/min to 240 °C. The final temperature of 240 °C was held for 20 minutes. The SIM programme meant that at any time during an analysis the mass spectrometer was searching for 4 specific mass values, two for each of two chlorination levels, Section 2.1.2. All of the breast milk samples were analysed in duplicate, and the average results were reported.

2.4.2: QUANTITATION OF PCBS:

The same method for quantifying the PCBs in the extracts was used as in Section 2.1.2. The quantitation of the PCBs present was achieved by using an external calibration standard called RPCBR-1 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), which contained two mono-substituted PCB congeners, and one congener for each of the other nine chlorination levels. The standard solution, supplied by Greyhound, contained eleven individual PCB congeners, each at a concentration of 1000 μg/ml. The eleven PCBs present were No.s 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, 209. Each PCB congener selected by the company had a GC-MS response factor which was representative of the response factors of the other PCB congeners of that chlorination level.

A set of solutions of this RPCBR-1 standard at different concentrations were made up and run on the GC-MS using the SIM programme specially developed for PCBs. Six calibration solutions were prepared, containing the PCB congeners at concentrations of 5 μg/ml, 1 μg/ml, 500 ng/ml, 250 ng/ml, 100 ng/ml, and 50 ng/ml, respectively. All of the standards had 4,4'-dibromobiphenyl present as an internal standard at a concentration of 1 μg/ml (or 1 ppm), exactly as in the breast milk extracts. The six standard solutions were run on the GC-MS.

Calibration curves for each of the ten chlorination levels were constructed using a computer, with the peak area of the PCB congener of interest divided by the peak area of the internal standard (4,4'-dibromobiphenyl), and plotted against the known concentrations of the RPCBR-1 solutions.

These ten calibration curves were then used to calculate the amounts of PCBs in the breast milk samples by chlorination level (homologue). The use of an internal standard allowed for any variation in the actual volume of sample injected by the GC autosampler. Each breast milk extract was analysed in duplicate, results were calculated by homologue, and the average results have been reported.

Calibration curves for each of the four ^{13}C-labelled PCBs were constructed by running 4 solutions of the SS001 standard (United States Environmental Protection Agency, Research Triangle Park, U.S.A.) at different concentrations, with 4,4'-dibromobiphenyl present in all the solutions, at a concentration of 1 μg/ml. The 4 solutions were made by diluting the SS001 standard by 1 in 5, 1 in 10, 2 in 25, and 1 in 20 using the appropriate volumetric flasks.

The peak areas of the four ^{13}C-labelled PCBs in the recovery standard were divided by the peak area of the internal standard, and calibration curves for each congener were drawn by plotting this ratio against the known concentrations of the SS001 solutions on a computer. The experimental percentage recovery of each individual breast milk sample could then be calculated.

Each final breast milk extract had 100 μl of the SS001 standard added to it initially. The final volume of each extract was 1 ml, and therefore the extracts should contain a 1 in 10 dilution of the SS001 standard, assuming a 100 % experimental recovery. The actual experimental percentage recoveries were calculated by comparison of the experimental peak height ratios of the ^{13}C-labelled PCBs with their calibration curves. The ^{13}C-labelled PCB congener No.212, ($^{13}C_{12}$) 3, 3', 4, 4'-tetrachlorobiphenyl, was used to calculate the experimental percentage recoveries of the extracts. This was because, as a tetrachlorobiphenyl, its behaviour most closely matched that of the majority of PCB congeners found in the breast milk extracts.

CHAPTER 3: THE DETERMINATION OF PCBS IN FISH OIL SAMPLES

3.1: INTRODUCTION:

It has been well documented that fish contain fairly high concentrations of PCBs, due to their position near to the top of the marine food chain. It has also been shown that any PCBs present in a fish would tend to accumulate in the fatty tissue, and, therefore, fish oil preparations would be expected to contain relatively high levels of PCBs. The fact that over 60 % of the world environmental PCB load is contained in oceanic waters (4), and that fish form a substantial part of the daily diet in many parts of the world make the analysis of fish and fish oils an important part of the continuing monitoring of PCBs to which humans may be exposed.

The analysis of fish oils for PCBs requires the separation of the PCBs from the fish oil matrix, prior to the determination of the PCBs by gas chromatography. The separation and removal of the oil has usually been carried out by low resolution column chromatography using an adsorbent such as silica, alumina, or Florisil as the stationary phase. Gel permeation chromatography and HPLC have also been used for this purpose.

Most of the research carried out in this area has involved the determination of PCBs in fish samples rather than commercial fish oil samples. A wide range of researchers have calculated PCB levels in a variety of types of fish, and in a number of different parts of the fish. There have been very few investigations into the levels of PCBs present in commercially available fish oil products. The work of several research groups into the levels of PCBs found in fish are detailed below.

3.1.1: LEVELS OF PCBS IN FISH:

Musial and Uthe (75) reported on the levels of PCBs found in herring oil from Canada. The oil was extracted from herring fillets with acetone, and then hexane. Aroclor 1254 at a concentration of approximately 1 mg/kg oil was added to some of the extracts. The PCBs were separated from the lipids by column chromatography. The column used contained Florisil activated at 450 ^{o}C overnight and deactivated with 1 % water. The Florisil column was then washed with hexane, before the oil was added onto the column. The PCBs were analysed by packed column GC-ECD, and quantified using the same Aroclor 1254 as had been previously added to the extracts. Musial and Uthe compared levels of Aroclor 1254 found in spiked and unspiked herring oil samples stored for different periods of time over an eight year timespan. The levels of PCBs found in the unspiked herring oil samples dropped slightly over the eight years from approximately 1.4 mg/kg oil to approximately 1.2 mg/kg oil. The PCBs were quantitatively recovered from the spiked herring oil samples over the eight year period. These results showed that fish oils could be stored and subsequently analysed over a long period of time.

Zitko *et al.* (76) analysed PCBs in 2 lots of 24 herring samples and 2 lots of 25 perch samples from eastern Canada. The PCBs were extracted from the muscle of the fish by Soxhlet extraction with hexane for 1 hour, and cleaned up by alumina column chromatography, followed by silica column chromatography. The extracts were analysed by packed column GC-ECD, and quantified using an Aroclor 1254 standard. The two lots of herring samples were found to contain average amounts of 0.2 mg/kg and 0.5 mg/kg wet weight of PCBs, while the two lots of perch samples were found to contain 0.03 mg/kg and 0.05 mg/kg wet weight of PCBs.

Tausch *et al.* (77) analysed the level of PCBs in a fish called a Rapfen from the Austrian section of the River Danube. The whole fish sample was extracted by centrifuging in petroleum ether, and then a Florisil column chromatography clean-up step was used. The extract was analysed for PCBs on a capillary GC-MS, and Aroclor 1254 was used as the quantitation standard. 40 PCB congener peaks were quantified in the extract, ranging from trichlorobiphenyls

to heptachlorobiphenyls. The Total PCB concentration found in the whole fish extract was 6.08 mg/kg.

Price *et al.* (78) analysed a blended sample of three lake trout from Lake Michigan, U.S.A. for PCBs. The fish sample was mixed with anhydrous sodium sulphate, and packed into a glass chromatography column. The sample was extracted with petroleum ether. The extract was then cleaned up using a Florisil column, followed by a silica column. The extract was analysed for PCBs and pesticides by packed column GC-ECD, and the PCBs were quantified using an Aroclor 1254 standard. Ten replicate analyses of the fish sample yielded a mean Total PCB concentration of 1.2 mg/kg whole weight, with a relative standard deviation of 2.4 %.

Grob, Jr. *et al.* (79) used a coupled HPLC-GC to determine PCBs in fish samples from Switzerland. The fish samples were extracted into petroleum ether using a Soxhlet apparatus. The petroleum ether was evaporated to dryness, and the extracts were reconstituted in n-pentane. The extracts were injected onto a 100 x 3 mm i.d. Spherisorb-S-5-CN HPLC column which was coupled to a capillary column GC-ECD. The HPLC mobile phase used was n-pentane. The PCB fraction from the HPLC, with a volume of 400 µl, was transferred directly to the GC column. Total PCB concentrations in the fish samples analysed varied between 0.03 mg/kg and 0.9 mg/kg.

Wells *et al.* (80) carried out an inter-laboratory programme to reduce the variation in the reported results of standard PCB congeners. The different laboratories involved were sent sewage, eel, and mussel samples with known concentrations of PCBs, and told to analyse the samples. The different laboratories were then asked to complete a carefully designed programme to improve the determination of PCBs. Finally, the laboratories were asked to measure the levels of seven individual PCB congeners in cod, herring, and mackerel oil samples. The seven congeners used were PCBs No. 28, 52, 101, 118, 138, 153, and 180. The analyses were carried out on capillary column GC-ECDs. Levels of the seven PCB congeners were found at between 50 ng/kg and 1000 ng/kg in the fish oils. PCB No. 118 was found at an average level of 500 ng/kg in the mackerel oil.

Maack and Sonzogni (81) analysed PCBs in a number of different fish samples from the U.S.A. The fish species analysed included lake chub, whitefish, sturgeon, whitebass, catfish, carp,

and walleye. The fish tissue samples were ground and column extracted with dichloromethane. The extracts were cleaned up by automated gel permeation chromatography, followed by silica gel fractionation. Capillary column GC-ECD was used to analyse the extracts. The quantification of identifiable PCB congeners was achieved by using Aroclors 1242, 1248, 1254, 1260, and 1262 as standards. The concentrations of Total PCBs found, determined by adding individual congener concentrations, ranged from 0.07 mg/kg to 7.0 mg/kg. The mean Total PCB concentration was 1.3 mg/kg. No correlation was observed for Total PCB concentrations and the species or location of the fish.

Bush *et al.* (82) analysed the levels of PCBs in 60 striped bass from five different sites in New York State, U.S.A. The fish samples were ground with sodium sulphate and 10 ml of hexane in a tissuemizer. Next, the volume of hexane was reduced using a Kuderna-Danish apparatus. The extracts were cleaned up by Florisil column chromatography, and analysed by capillary column GC-ECD. The extracts were quantified by using a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254, and 1260. The average concentrations of Total PCBs found at four of the five sites ranged from 7.5 mg/kg to 1.8 mg/kg, while the average Total PCB concentration found in the samples from Hudson Bay was 15 mg/kg. The Hudson Bay area had been polluted with PCBs from industrial manufacturing plants over a long period of time.

Li *et al.* (83) measured PCBs in fish from the Second Shonghua River in the People's Republic of China. Fish muscle, fish organs, and whole young fish were all analysed. The Total PCBs found in fish muscle ranged from 6.4 μg/kg to 71.2 μg/kg. The Total PCBs found in fish organs ranged from 5.4 μg/kg to 613.2 μg/kg. The Total PCBs found in whole fish ranged from 118.6 μg/kg to 214.5 μg/kg. The concentration of PCBs was, therefore, higher in the fish organ samples than in the fish muscle samples.

Tuinstra *et al.* (84) determined the levels of specific PCB congeners in fish samples from the Netherlands. 33 eels from the River Rhine were analysed. The eels were blended, chloroform was added, and the samples were centrifuged. The resultant extracts were cleaned up by gel permeation chromatography, and analysed by capillary column GC-ECD. A standard solution containing 24

individual PCB congeners was used for quantitation. The Total PCB content of the eels was calculated using this standard solution. The Total PCB content of the 33 eel samples varied from 0.2 mg/kg to 12 mg/kg, with a mean of 5.86 mg/kg. The levels of six individual PCB congeners (PCB No. 28, 52, 101, 138, 153, 180) were reported in each of the 33 eel samples. The individual congeners were found at concentrations between 0.005 mg/kg and 1.2 mg/kg. These six congeners were among the most prevalent PCBs previously found in a whole range of sample matrices.

Mes *et al.* (43) analysed the levels of 34 selected PCB congeners in a number of fish products, as part of a wider ranging survey of PCB levels in the Canadian diet. Samples of fish were extracted using an homogeniser, and the extracts filtered. Gel permation chromatography was used as the clean-up step, followed by a Florisil column chromatography step. A capillary column GC-ECD was used to analyse the extracts. Capillary column GC-MS was also used to confirm the identities of the peaks observed on the GC-ECD. A standard solution containing known concentrations of the 34 PCB congeners of interest was used as the quantitation standard. The mean Total PCB residue levels found in the fish samples, calculated by summing the amounts found for the 34 individual PCB congeners, were 3.2 μg/kg in marine fish, 21.0 μg/kg in freshwater fish, 2.3 μg/kg in shellfish, and 11.5 μg/kg in canned fish. All of these results were expressed on a wet weight basis. The individual levels of the 34 PCB congeners in all of the fish samples were also reported. The Total PCB levels found in the freshwater fish and canned fish samples were higher than those found in a wide range of different foodstuffs, such as meat and dairy products. The levels in the fish were, however, lower than the Canadian government guidelines for Total PCBs of 2000 μg/kg edible part.

Tanabe *et al.* (85) reported the levels of toxic coplanar PCBs in striped mullet, as well as other marine and terrestrial creatures. The fish samples were saponified in ethanolic potassium hydroxide, and extracted. An activated charcoal column was used to separate the coplanar PCBs from co-extractants. The extracts were then cleaned up with fuming sulphuric acid (oleum), ready for GC analysis. Capillary column GC-ECD was used for quantitation, and capillary column GC-MS was used for confirmation of peak identities. A standard solution containing the three PCB

congeners of interest (PCBs No. 77, 126, 169) was used for quantitation. The striped mullet samples were found to contain average amounts of 2100 ng/kg of PCB No. 77, 100 ng/kg of PCB No. 126, and 2.4 ng/kg of PCB No. 169. An average Total PCB content of 1.2 mg/kg was also calculated. All of these results were expressed on a wet weight basis.

Mes and Weber (44) also reported the levels of non-orthochlorine substituted coplanar PCB congeners in two fish products, as part of a wider survey of levels of these specific PCB congeners in the Canadian diet. The samples were saponified with ethanolic potassium hydroxide, packed into a column, and extracted with hexane. Charcoal column chromatography was used to separate the coplanar PCBs from the other extracted compounds. The extract was cleaned up using 5 % fuming sulphuric acid, ready for GC analysis. Capillary column GC-ECD was used to quantify the coplanar PCBs, and capillary column GC-MS was used to confirm the identities of the coplanar PCBs. A standard solution containing the three coplanar PCB congeners of interest (PCBs No. 77, 126, 169) was used for quantitation. Freshwater fish were found to contain average levels of 36 ng/kg of PCB No. 77, 8 ng/kg of PCB No. 126, and <1 ng/kg of PCB No. 169. Canned fish were found to contain average levels of 8 ng/kg of PCB No. 77, 3 ng/kg of PCB No. 126, and < 1 ng/kg of PCB No. 169. All of these results were expressed on a wet weight basis. The PCB levels in the freshwater fish were higher than those in any of the other sample matrices examined in the study, e.g. meat, milk.

3.2: RESULTS:

A number of commercially available fish liver oil and fish oil products were purchased through normal retail outlets such as pharmacies and health food stores. They may be classified as either fish liver oil preparations recommended for their content of Vitamins A, D, and E, or as fish oil concentrates containing the polyunsaturated fatty acids Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). As a result of widespread publicity campaigns many different types of people now take these products, some because of worries about possible vitamin deficiencies,

and others as part of a so-called "healthy" lifestyle. The recommended daily doses of these fish oil products can be quite large, and it was intended, therefore, to determine exactly what levels of PCBs may be ingested by pregnant women, small children or the elderly through the taking of such fish oil products. Only one previous study of the levels of PCBs in fish oil products in Great Britain had been reported, published by the Ministry of Agriculture, Fisheries and Food in 1983 (86). This report gave results for the analyses of four samples of bottled cod liver oil, two of halibut liver oil capsules, and one of bottled halibut liver oil.

For this study, eight different fish oil and fish liver oil products were purchased in total. Three different brands of fish oil products were investigated, along with five different brands of fish liver oil products. The results for the eight different products are shown in Table 3.1. All of the extracts were analysed in duplicate, and the average results are shown. The results have been split into the five fish liver oil products and the three fish oil products, to examine the differences and similarities in the experimental results found for the two sets of samples.

PRODUCT NAME	PCB COMPOSITION BY HOMOLOGUE (mg/kg oil)						TOTAL PCB CONC (mg/kg oil)
	2Cl	3Cl	4Cl	5Cl	6Cl	7Cl	
Halibut Liver Oil							
Capsules							
Crookes	0.0035	0.0276	0.0133	0.0185	0.0292	0.0045	0.0966
Boots	ND	0.0068	0.0175	0.0182	0.0315	0.0017	0.0757
Cod Liver Oil							
Capsules							
Sanatogen	ND	0.0123	0.0106	0.0322	0.0202	0.0048	0.0801
Seven Seas	0.0011	0.0095	0.0171	0.0238	0.0548	0.0056	0.1119
Cod Liver Oil							
B.P. Liquid							
Boots	0.0017	0.0119	0.0142	0.0232	0.0604	0.0060	0.1174
Fish Oils							
Capsules							
Sanatogen ("Omega 3")	0.0037	0.0191	0.0223	0.0032	0.0006	ND	0.0489
Boots ("EPA")	0.0027	0.0211	0.0198	0.0024	0.0005	ND	0.0465
Seven Seas ("Pulse")	0.0011	0.0195	0.0148	0.0017	0.0015	ND	0.0386

ND = not detected.

TABLE 3.1: CONTENT AND COMPOSITION OF PCBS IDENTIFIED IN FISH LIVER OIL AND FISH OIL SAMPLES

The Total PCB results for all of the five fish liver oil products were similar and ranged from 0.0757 mg/kg oil to 0.1174 mg/kg oil, Table 3.1. The halibut liver oil contained an average Total PCB level of 0.0862 mg/kg oil, and the cod liver oil contained an average Total PCB level of 0.1031 mg/kg oil. Trichlorobiphenyls through to heptachlorobiphenyls were found in all five fish liver oil products, with pentachlorobiphenyls and hexachlorobiphenyls being the most abundant. Dichlorobiphenyls were found in the Crookes HLO capsules, Seven Seas CLO capsules, and Boots CLO B.P. liquid, but not in the other two products. The Total PCB levels were much lower than those reported for fish liver oil samples in the 1983 report (86), which found PCB levels of about 1 mg/kg oil in cod liver oil (4 samples) and 0.7 mg/kg in halibut liver oil (2 samples). One sample of

bottled halibut liver oil contained 7 mg/kg oil. The Total PCB levels found in this work were only about 10 % of the values in this 1983 report (86).

The composition by homologue of the PCBs identified in the five halibut and cod liver oils were very similar both in terms of their chlorination profiles and the total amounts present. This can be clearly seen in Diagram 3.1, where the levels of PCBs found in the five fish liver oil samples are represented as a bar chart.

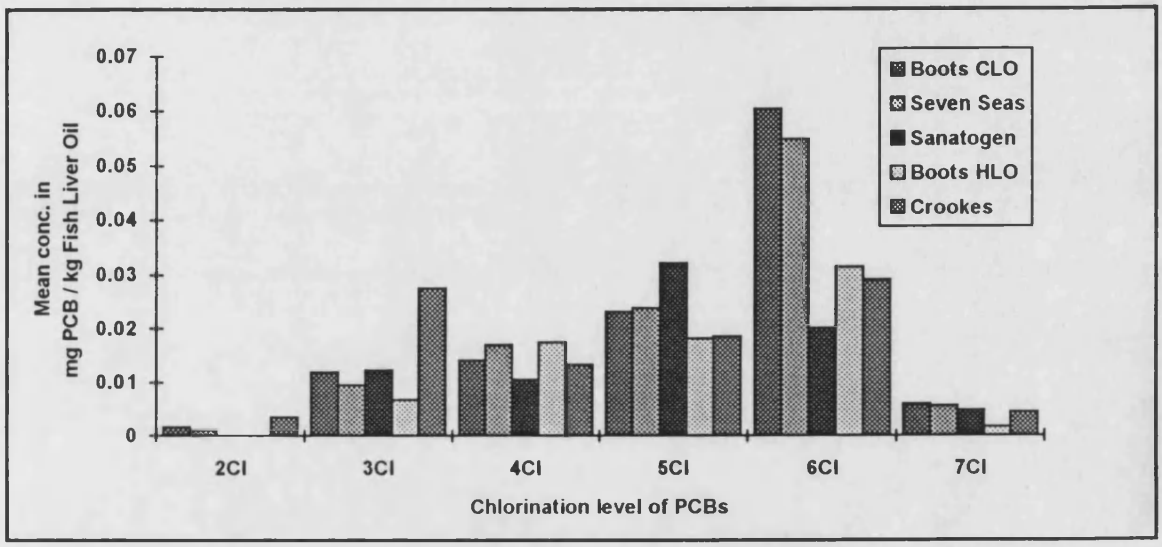


DIAGRAM 3.1: COMPARISON OF THE CONCENTRATIONS OF PCBS FOUND IN 5 DIFFERENT FISH LIVER OIL PRODUCTS

The three fish oil products were found to contain Total PCB levels ranging between 0.0386 mg/kg oil and 0.0489 mg/kg oil, with an average Total PCB level of 0.0421 mg/kg oil, Table 3.1. These results were less than half the values found in the fish liver oil products. This was in agreement with the work of Li *et al.* (83) who found higher levels of PCBs in fish organs, including livers, than in other parts of the fish. Dichlorobiphenyls through to hexachlorobiphenyls were found in all three samples, with trichlorobiphenyls and tetrachlorobiphenyls being the most abundant.

The composition by homologue of the PCBs identified in the three fish oil products were very similar both in terms of their chlorination profiles and the total amounts present. The levels of PCBs found in the fish oils can be seen in Diagram 3.2, represented in the same way as the fish liver oils results in Diagram 3.1.

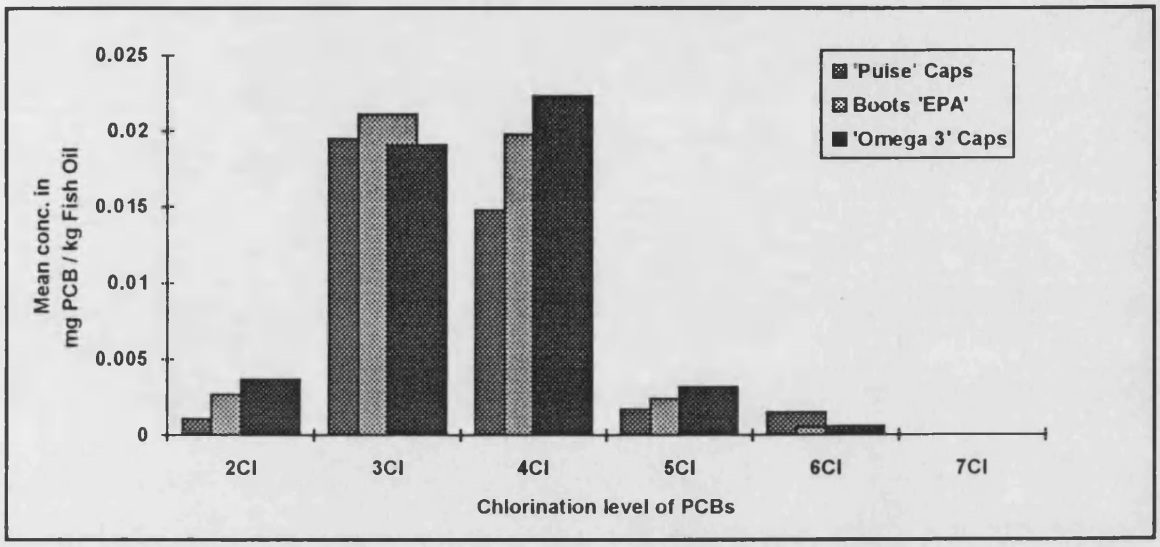


DIAGRAM 3.2: COMPARISON OF THE CONCENTRATIONS OF PCBS FOUND IN 3 DIFFERENT FISH OIL PRODUCTS

The chlorination profiles obtained for the fish liver oils were very different from those found in the fish oils, which can be seen by comparing Diagram 3.1 and Diagram 3.2. The most abundant PCBs in the fish oils contained 3 or 4 chlorine atoms, whereas those in the fish liver oils contained 5 or 6 chlorine atoms.

The average levels of PCBs in mg/kg oil found in the five fish liver oils and the three fish oils were directly compared in Table 3.2.

TYPE OF OIL SAMPLE	MEAN PCB CONCENTRATION (mg/kg oil)					
	2Cl	3Cl	4Cl	5Cl	6Cl	7Cl
Fish Liver Oils (n = 5)	0.0013	0.0136	0.0145	0.0232	0.0271	0.0045
Fish Oils (n = 3)	0.0025	0.0199	0.0190	0.0024	0.0008	ND

ND = not detected.

TABLE 3.2: COMPARISON OF THE AVERAGE PCB CONCENTRATIONS DETECTED IN FISH LIVER OIL AND FISH OIL SAMPLES

It can be seen that the major difference between the results for the fish liver oils and those for the fish oils was the much higher levels of pentachlorobiphenyls and hexachlorobiphenyls found in the fish liver oils. The results for the levels of the other PCB homologues in the two types of oils were very similar. Pentachlorobiphenyls and hexachlorobiphenyls are metabolised more slowly than trichlorobiphenyls and tetrachlorobiphenyls by fish (85). The results indicate that some metabolism has occurred in the fish liver oil samples, while less metabolism has occurred in the fish oil samples. The precise source of the fish oil products was not given on the bottles, but the information that was supplied suggested that the oils were obtained from various tissues of selected fish species. The differences between the average results for the fish liver oil samples and the fish oil samples are represented graphically in Diagram 3.3.

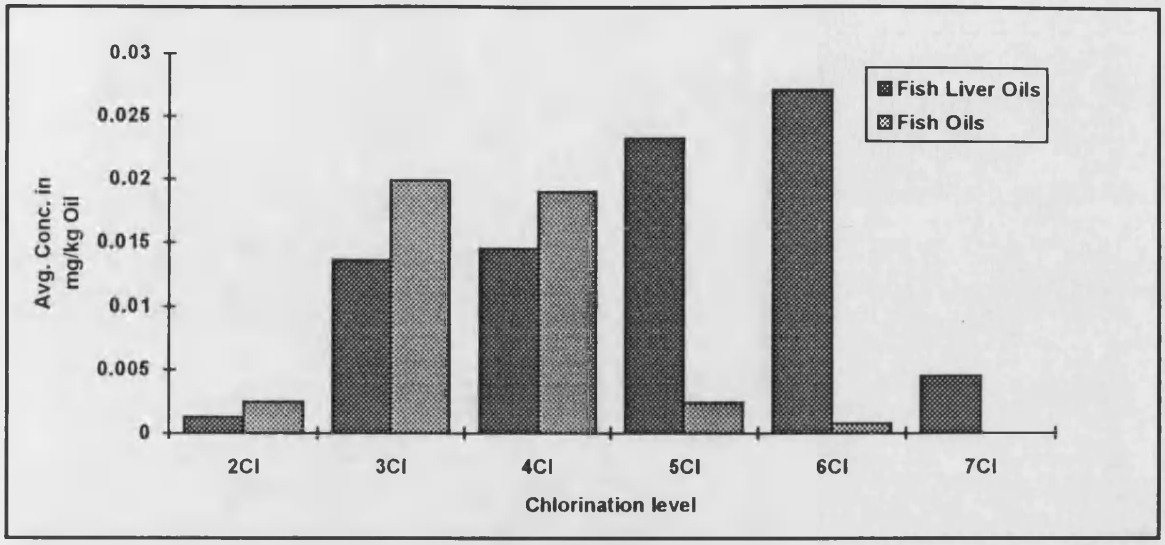


DIAGRAM 3.3: COMPARISON OF THE AVERAGE PCB CONCENTRATIONS DETECTED IN FISH LIVER OIL AND FISH OIL SAMPLES

The average experimental percentage recoveries for duplicate extractions of the eight samples ranged from 49.6 % to 76.5 %. The percentage recoveries of the four ^{13}C-labelled PCB congeners were taken into account in calculating each of the PCB results in Table 3.1.

3.2.1: DAILY INTAKE OF PCBS:

The contributions that these fish products would make to the daily intake of PCBs by a human being were calculated using the recommended daily doses given by the manufacturers on the bottles, Table 3.3. The total weight of oil in a capsule was measured in three capsules for each different product, and an average calculated. The results for the daily intake of PCBs for each product, calculated using the daily doses and total weights of oil, are summarised in Table 3.3.

PRODUCT NAME	WEIGHT OF OIL IN CAPSULE (mg)	RECOMMENDED DAILY DOSE	WEIGHT OF PCBS IN DAILY DOSE (μg)
Halibut Liver Oil			
Capsules			
Crookes	164	One Capsule	0.015
Boots	159	One Capsule	0.012
Cod Liver Oil			
Capsules			
Sanatogen	293	Three Capsules	0.070
Seven Seas	321	Six Capsules	0.220
Cod Liver Oil			
B.P. Liquid			
Boots	0.92g/ml	5ml	0.540
Fish Oils			
Capsules			
Sanatogen ("Omega 3")	494	Two Capsules	0.049
Boots ("EPA")	979	Four Capsules	0.18
Seven Seas ("Pulse")	523	Two Capsules	0.041

TABLE 3.3: DAILY INTAKES OF PCBS FROM FOLLOWING RECOMMENDED DOSES OF THE FISH LIVER OIL AND FISH OIL PRODUCTS

This shows that the average daily intake of PCBs from halibut liver oil capsules was 0.013 μg, whereas the higher recommended daily doses of the cod liver oil capsules meant that the average daily intake of PCBs from them was higher at 0.145 μg. The daily dose of the bottled cod liver oil of 5 ml, which was higher than any of the capsules, meant that the average daily intake of PCBs from it was higher at 0.54 μg.

Although the three fish oil products had recommended daily doses that contained larger volumes of oil than the fish liver oil products, their lower Total PCB levels meant that the average daily intake of PCBs from the fish oil products was only 0.09 μg.

All of the values for the daily intake of PCBs from the eight samples were small, compared with a value of between 1 μg/day and 7 μg/day in the 1983 MAFF report (86). The ingestion of

fish oils is unlikely to be a major source of PCB intake in the general population, whereas the eating of fish is a major source of PCB intake (86).

3.3: DISCUSSION:

The work presented demonstrates that HPLC with a 300 mm Polymer PLRP-S column can successfully separate lipids from PCBs and other organic compounds in fish liver oil and fish oil samples. HPLC with a mobile phase of 60 % heptane and 40 % propan-2-ol at a flowrate of 2.3 ml/min can separate and elute the lipids and the PCBs in 17 minutes. This is faster than most column chromatography or gel permeation chromatography methods. The final extract is in a volume of less than 25 ml, lower than most previously reported methods, thus reducing the time required to prepare the final extracts for GC analysis. The HPLC column is also easy to maintain, by washing with a 100 % heptane mobile phase for 15 minutes between runs.

PCBs have been detected in all eight of the fish liver oil and fish oil samples that were analysed. Trichlorobiphenyls through to hexachlorobiphenyls have been detected in all of the samples, and dichlorobiphenyls and heptachlorobiphenyls have been detected in some of the samples. The five fish liver oil products contained an average Total PCB concentration of 0.0784 mg/kg oil, and the three fish oil products contained an average Total PCB concentration of 0.0421 mg/kg oil.

Although the actual levels of Total PCBs found were not higher than those found by other researchers, it should still be of concern that products specifically designed to be taken by people who are at a greater risk of illness than the general population, i.e. pregnant women, children, the elderly, should contain appreciable levels of organic pollutants. The consumption of these products for a lengthy period of time could lead to the ingestion of fairly large quantities of PCBs. These PCBs would be stored in the body, and not excreted.

The work presented in this chapter is only designed to be a small scale investigation into the levels of PCBs present in fish oils. A larger study of samples from a wider range of fish-based

products consumed in the daily diet would need to be carried out to discover how the levels of PCBs present vary with the types of fish, and parts of the fish used to make these commercial products.

CHAPTER 4: THE DETERMINATION OF PCBS IN EGG SAMPLES

4.1: INTRODUCTION:

PCBs have been detected in eggs from a variety of different bird species. The many species of birds worldwide can come into contact with PCBs in a number of ways. Many species of birds are fish eaters, and therefore appear near the top of marine food chains. PCBs ingested by plankton and other small marine species bioaccumulate in the fatty regions of the fish that eat them. The PCBs are then transferred to the birds upon eating the fish. The PCBs then tend to bioaccumulate in the fatty tissues of the birds.

Farmyard birds such as chickens can become exposed to PCBs in other ways. The feed, water, or litter used by the farmer may contain PCBs. Other types of organochlorine compounds used on the farms for cleaning buildings, spraying crops, or topical treatment of the birds may contain PCBs as contaminants. In recent years, the use of organochlorines on farms in Europe and North America has been reduced because of public health worries.

Female birds can only eliminate the PCBs from their bodies in faeces or in the eggs that they produce. Eggs are an important part of the daily diet in many parts of the world and, therefore, the levels of PCBs present in them have been widely investigated over the last 20 years.

4.1.1: LEVELS OF PCBS IN EGGS:

Zitko (87) reported the levels of PCBs in the eggs of double-crested cormorants in Canada between 1971 and 1975. The eggs were boiled and then stored frozen until analysis. For analysis, the yolks of the eggs were ground with anhydrous sodium sulphate, and extracted with hexane in a Soxhlet extractor for 1 hour. Alumina column chromatography was used to separate the lipids from the organic extractants, followed by a silica column chromatography step to separate the PCBs from the pesticides. Packed column GC-ECD was used to analyse the extracts, and the PCBs were quantified using Aroclor 1254 as a standard. Packed column GC-MS was used to

confirm the identities of the PCB peaks. Eggs were analysed for each of the years between 1971 and 1975, with between 9 and 11 egg samples analysed per year. Total PCBs, expressed as Aroclor 1254, were found at levels from 5.23 μg/g wet weight to 14.3 μg/g wet weight. It was found that there was a decrease in the Total PCB levels between 1971 and 1973, and no change thereafter. Some problems with the suitability of Aroclor 1254 as the quantitation standard were expressed, because the pattern of PCB peaks found in the eggs was markedly different from the pattern of Aroclor 1254.

Kveseth and Brevik (88) reported the analysis of a contaminated seabird egg from Norway. They used a mixed alumina and silica gel column to separate lipids and pesticides from PCBs simultaneously, after the initial extraction step. Hexane was used as the eluting solvent. Packed column GC-ECD was used to analyse the PCBs, with Clophen A50 used as the quantitation standard. A Total PCB level of 88 μg/g on a fat basis was found in the seabird egg.

Frank *et al.* (89) analysed the levels of PCBs, and other organochlorine residues, in chicken eggs from Ontario, Canada between 1969 and 1982. The eggs were mixed thoroughly with anhydrous sodium sulphate, and exhaustively extracted with hexane in a Soxhlet apparatus. Florisil column chromatography was used to clean-up the samples, followed by a silicic acid column chromatography step. The analysis of the PCBs present was carried out on a packed column GC-ECD, with mixtures of Aroclors 1254 and 1260 used for identification and quantitation. Between 1969 and 1982, a total of 118 composite samples were analysed representing 419 eggs. PCB residues declined in the egg samples from 405 ng/g fat in 1969-70 to less than 10 ng/g fat in 1981-82. These changes reflected the action taken to curb the use of PCBs over this period. The levels found in the chicken eggs were much lower than those found in many seabird eggs, due to the differences in diet and habitat.

Driss and Bouguerra (90) determined the levels of PCBs in 33 falcon eggs from Tunisia. The yolk of each egg was carefully separated from the rest of the egg, and mixed with 20 g of anhydrous sodium sulphate. This was mixed in a blender with acetone, and then hexane. The extract was then filtered and washed. A Florisil column chromatography clean-up step, followed

by a sulphuric acid treatment step was used. Finally an alumina column was used to separate the PCBs from any pesticides. The extracts were analysed by packed column GC-ECD, and Phenochlor DP6 was used for quantitation. The Total PCB concentration, found by analysing 9 egg samples in duplicate, ranged from 6.3 μg/g to 89.5 μg/g on a fat basis.

Hernández *et al.* (91) analysed the levels of PCBs in a range of different bird eggs from Spain. A total of 69 eggs from eagles, kites, falcons, buzzards, storks, and spoonbills were analysed. The eggs were kept frozen until they were analysed. 3 grams of each egg sample were homogenised, mixed with anhydrous sodium sulphate, and extracted with hexane in a Soxhlet apparatus for 8 hours. The extracts were cleaned up by Florisil column chromatography, followed by silica gel chromatography. The extracts were analysed by GC with an electron affinity detector. PCBs were found in all of the 69 eggs analysed. The Total PCB concentration ranged from 0.14 μ g/g to 18.75 μg/g wet weight, and the means for each bird species ranged from 0.206 μg/g to 2.882 μg/g wet weight. The eggs had been collected from two distinct areas of Spain and the levels of PCBs in eggs from one region were found to be higher than in the other region. This was thought to be due to the use of insecticides, and the contamination of the main river in the region which showed the higher PCB concentrations.

The same research group (92) also reported on the levels of Total PCBs and some individual PCB congeners in Spanish Imperial Eagle Eggs. Exactly the same extraction and clean-up methodology was used as previously outlined (91). The extracts were analysed using a capillary column GC-ECD. Aroclor 1260 was used to quantify the Total PCBs present, while a standard solution containing PCBs No. 101, 118, 138, 153, and 180 was used to quantify these five PCB congeners. A total of 34 eggs from three different regions of Spain were analysed, and PCBs were found in all of them. The Total PCB concentration found ranged from 115 ng/g to 28,917 ng/g wet weight, with means of 293 ng/g, 280 ng/g, and 820 ng/g wet weight for the three regions. All 34 of the eggs contained PCBs No. 101, 153, and 180. PCB No. 118 was detected in 9 eggs, and PCB No. 138 was detected in 32 eggs. Again, eggs from one region contained higher PCB levels than

the other regions. The same region as in the previous reference (90) gave the highest PCB levels, for the same reasons.

Frank and Braun (93) reported the levels of PCBs and other organochlorine compounds in a wide range of birds and bird eggs from Ontario, Canada. The eggs were mixed with anhydrous sodium sulphate and extracted with hexane in a Soxhlet apparatus. Florisil column chromatography was used to clean-up the egg extracts, followed by a charcoal column chromatography step to separate the PCBs from other organochlorine compounds. The extracts were analysed by packed column GC-ECD. A mixture of Aroclors 1254 and 1260 was used for quantitation. The birds were categorised into different groups by the authors. 6 eggs from birds feeding in an aquatic environment, i.e. ducks and swans, were analysed. Total PCB levels of between 0.1 μg/g and 0.4 μg/g wet weight were found in the six eggs. 15 eggs from gulls and terns were analysed, 3 from gulls and 12 from terns. The gulls eggs were found to contain an average Total PCB level of 60 μg/g wet weight, and the tern eggs were found to contain an average Total PCB level of 2.6 μg/g wet weight.

Perry *et al.* (73) analysed PCBs in egret and heron eggs from Israel. Egrets and herons are both fish-eating bird species. The eggs were frozen on collection. Each egg was homogenised in a blender, and ground with anhydrous sodium sulphate to yield a dry flowing powder. The eggs were extracted in a Soxhlet apparatus for 2 hours with a 1:4 mixture of acetone:hexane. The extracts were cleaned up by Florisil column chromatography, and the PCBs separated from the organochlorine pesticides using a silicic acid column. The PCBs were perchlorinated with antimony pentachloride, and the resultant decachlorobiphenyl was quantitated against Aroclor 1254, which was treated in the same manner, or against a decachlorobiphenyl standard. Analysis of 8 egret eggs gave a mean Total PCB concentration of 0.54 μg/g +/-0.28 μg/g wet weight, while analysis of 10 heron eggs gave a mean Total PCB concentration of 0.77 μg/g +/-0.64 μg/g wet weight.

Driss *et al.* (94) analysed the levels of PCBs in falcon and pigeon eggs from Tunisia. The eggs were extracted and cleaned up using the same methodology as reported previously (90). The

extracts were analysed by capillary column GC-ECD, and a standard of Phenochlor DP6 was used for quantitation. A total of 17 individual PCB congeners were identified from the GC-ECD trace of the standard, and levels for these congeners were reported for each egg extract. 3 falcon egg samples and 3 pigeon egg samples were analysed. The Total PCB concentration for each extract was reported, as well as the individual congener results. The Total PCB concentration ranged from 20.33 μg/g to 127.2 μg/g for the falcon eggs, and from 0.62 μg/g to 3.217 μg/g for the pigeon eggs. All of the egg samples were found to have relatively high amounts of the individual congeners PCBs No. 138, 153, 170, and 180.

Mullié *et al.* (95) investigated the levels of organochlorine compounds and mercury in Cattle Egret eggs from the Faiyum Oasis in Egypt. Cattle Egrets are not piscivores, unlike most other egrets, but mainly insectivores, feeding in agricultural areas. 7 eggs were collected and freeze-dried. An aliquot of the freeze-dried egg was extracted with hexane for 12 hours in a Soxhlet apparatus. The extract was cleaned up by treating with sulphuric acid, followed by a Florisil column chromatography step. Subsequently, the PCBs were separated from other organochlorine compounds by a silica column chromatography step. The extracts were analysed by packed column GC-ECD. The Total PCB levels found in the eggs ranged from 0.177 μg/g to 1.587 μg/g dry weight, with a mean value of 0.609 μg/g +/-0.477 μg/g dry weight.

Frank *et al.* (96) reported the levels of PCBs found in hen's eggs from Ontario, Canada in 1986. The eggs were extracted with hexane using a Soxhlet apparatus, and cleaned up by Florisil column chromatography. The extracts were fractionated by charcoal column chromatography. The extracts were then analysed by packed column GC-ECD. Aroclors 1254 and 1260, individually or as a mixture, were used to quantify the PCBs found. A mean Total PCB level of less than 0.3 ng/g wet weight was found in the hen's eggs.

Brunn *et al.* (97) analysed the levels of PCBs present in a range of foodstuffs, including eggs, in the German daily diet. A total of 30 eggs were analysed. The eggs were homogenised, and Soxhlet extracted. The PCBs were separated from the fat by alumina column chromatography. The extracts were analysed by capillary column GC. Each sample was analysed

in duplicate on two different columns. The levels of 7 individual PCB congeners, PCBs No. 28, 49, 52, 101, 138, 153, 180, were reported. All of the congeners except PCB No. 28 were found in the egg samples, with PCBs No. 138, 153, and 180 present in the highest concentrations. The average levels of the individual congeners PCBs No. 138, 153, and 180 were 21 ng/g, 30 ng/g, and 14 ng/g fat, respectively. The average Total PCB concentration for the 7 individual congeners was 75 ng/g fat.

Mes *et al.* (43) determined the levels of 34 selected PCB congeners in some fatty foods present in the Canadian diet. Eggs were among the samples that were analysed. The eggs were cooked, ready for consumption, before being extracted. The egg samples were homogenised with a mixture of hexane and acetone, and filtered through a column containing anhydrous sodium sulphate to extract the compounds of interest. The lipids were separated from the PCBs by gel permeation chromatography. The eluents from the GPC step were then passed through a Florisil column chromatography step. Finally the extracts were treated with methanolic KOH, and eluted with hexane. The extracts were analysed by capillary column GC-ECD and capillary column GC-MS. Identification and quantification of the PCBs was done using the GC-ECD, while confirmation of peak identities was done using the GC-MS. A standard solution containing known concentrations of the 34 individual PCB congeners was used for quantitation. The Total PCB concentration found in the egg samples, calculated by summing the results for the 34 individual PCB congeners, was 0.7 ng/g wet weight. The levels of the 34 individual PCB congeners in the eggs in pg/g were also reported. The Canadian government's guideline for the maximum acceptable PCB concentration in eggs was quoted as 100 ng/g, so that the levels found were much lower than this limit.

Mes and Weber (44) analysed non-orthochlorine substituted coplanar PCB congeners in some fatty foods including eggs, as well as human fat and breast milk samples. The egg samples were refluxed with ethanolic KOH for 1 hour. The extracts were transferred to separating funnels containing hexane and water. After partitioning, the water phase was discarded, and the hexane fraction filtered through anhydrous sodium sulphate. A charcoal column chromatography step was

used to separate the coplanar PCBs from the other PCB congeners. Shaking with 5 % fuming sulphuric acid was used as a final clean-up step. The extracts were analysed using both capillary column GC-ECD and GC-MS. GC-ECD was used for the quantitation of the coplanar PCBs. A standard solution containing the three PCB congeners of interest, PCBs No. 77, 126, and 169, was used as the reference sample. All three of the coplanar PCB congeners were found in the egg samples, at a concentration of 1 pg/g wet weight for each of the three congeners.

4.2: RESULTS:

A total of 7 duck egg samples were analysed, as outlined in Chapter 2. The egg samples were analysed for 7 individual PCB congeners, using a capillary column GC-ECD. The results for the 7 individual PCB congeners, PCBs No. 28, 52, 101, 118, 138, 153, and 180, in each of the seven egg samples, are reported in Table 4.1. All of the egg extracts were run in duplicate on the GC-ECD, and the average concentrations of each PCB congener have been reported. In addition to the individual congener results, a Total PCB concentration for each egg sample was calculated by adding the results of the 7 individual PCB congeners together and multiplying by a factor of four, as specified by the ICES Method in Chapter 2.

PCB No.	Concentration of Congeners in ng/g Egg						
	EGG SAMPLES						
	KD	LF	LN	LO	LR	LS	LT
28	4.1	3.1	ND	2.7	6.4	5.3	2.7
52	ND	0.4	ND	1.6	0.9	0.8	0.1
101	ND	0.1	ND	0.1	0.2	ND	ND
118	2.1	0.5	0.1	0.1	0.1	0.2	0.1
138	3.2	1.2	0.2	0.3	1.1	0.3	0.1
153	3.4	1.3	0.3	0.3	1.5	0.4	0.1
180	1.2	0.5	0.2	0.2	0.9	0.1	0.1
Total PCBs	56.0	28.4	3.2	21.2	44.4	28.4	12.8

TABLE 4.1: CONCENTRATIONS OF PCBS FOUND IN THE 7 EGG SAMPLES

Table 4.1 shows that the egg samples were found to contain Total PCB concentrations of between 3.2 ng/g egg and 56.0 ng/g egg.

The mean Total PCB concentration for the 7 egg samples was calculated as 27.6 ng/g egg. The mean PCB concentrations for the 7 individual congeners in the 7 egg samples were calculated, and are shown in Table 4.2. These results are displayed graphically in Diagram 4.1.

PCB No.	Mean Conc. of PCB Congeners in ng/g Egg
28	3.5
52	0.5
101	0.06
118	0.5
138	0.9
153	1.0
180	0.5

TABLE 4.2: MEAN CONCENTRATIONS OF INDIVIDUAL PCB CONGENERS IN EGG SAMPLES

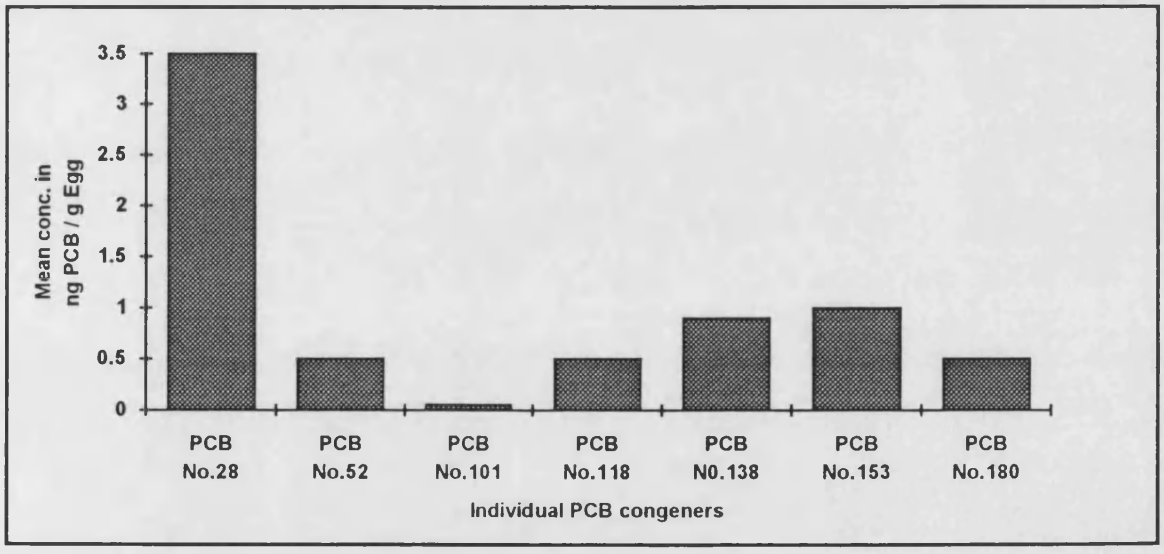


DIAGRAM 4.1: MEAN CONCENTRATIONS OF INDIVIDUAL PCB CONGENERS IN EGG SAMPLES

4.3 :DISCUSSION:

The finding of a mean Total PCB concentration of 27.6 ng/g egg for the egg samples was compared with the Total PCB concentrations found in various eggs by other researchers, Table 4.3.

REF No.	No. of Samples	Type of Bird	Mean Total PCB Conc. (ng/g ÷ 1000 wet wt.)
87. Zitko	48	Cormorants	5.23 - 14.3
91. Hernández *et al.*	69	Various	0.206 - 2.882
92. Hernández *et al.*	34	Imperial Eagles	0.29, 0.28, 0.82
93. Frank & Braun	6, 12, 3	Ducks & Swans, Terns, Gulls	0.23, 2.6, 60
73. Perry *et al.*	18	Egrets, Herons	0.54, 0.77
94. Driss *et al.*	6	Falcons, Pigeons	87.8, 1.8
96. Frank *et al.*	NA	Hens	$< 3 \times 10^{-4}$
43. Mes *et al.*	NA	Hens	7×10^{-4}
Current Work	7	Ducks	2.76×10^{-2}

TABLE 4.3: COMPARISON OF MEAN TOTAL PCB CONCENTRATIONS FOUND IN EGG SAMPLES

Table 4.3 shows that Frank & Braun (93) found a mean Total PCB concentration of 0.23 µg/g egg in duck and swan eggs from Canada. These egg extracts were analysed by packed column GC-ECD, and a mixture of Aroclors 1254 and 1260 was used for quantitation. This result is approximately 10 times higher than that found in the current work. The use of GC-ECD as the chromatographic technique by Frank & Braun, rather than GC-MS, might be one of the reasons why a higher PCB concentration was found by these researchers. The other reasons for the discrepancy in results include the difference in the quantitation methods used, and the differences between ducks and swans.

Frank *et al.* (96) found a mean Total PCB concentration of less than 0.3 ng/g egg in hen's eggs from Canada between 1985 and 1987, while Mes *et al.* (43) found a mean Total PCB concentration of 0.7 ng/g egg in hen's eggs from Canada in 1985-86. These results were much lower than those found in the current work. All of the other researchers listed in Table 4.3 analysed eggs from birds of prey or fish-eating birds, and found much higher Total PCB concentrations, as would be expected.

The concentrations of the 7 individual PCB congeners found in the present work and in two of the other references have been compared. The results for the 7 individual PCB congeners, PCB No. 28, 52, 101, 118, 138, 153, and 180, found in the current work, and by Hernández *et al.* (92) and Mes *et al.* (43) are shown in Table 4.4. In the present work, the results for the 7 egg samples have been averaged to give the mean results for the 7 individual PCB congeners.

REF No.	No. of Samples	Type of Bird	Concentration of PCB Congeners in ng/g wet wt.						
			PCB Congener No.						
			28	52	101	118	138	153	180
92. Hernández *et al.*	32	Imperial Eagles	ND	ND	13.4	10.1	22.4	58.3	56.3
43. Mes *et al.*	NA	Hens	0	0.029	ND	0.103	0.073	0.113	0.088
Current Work	7	Ducks	2.42	0.38	0.04	0.32	0.64	0.73	0.32

TABLE 4.4: COMPARISON OF INDIVIDUAL PCB CONGENERS FOUND IN EGG SAMPLES

Table 4.4 showed an order of magnitude difference in the actual concentrations of the individual PCB congeners found in the current work and by Mes *et al.* In addition, two other differences between these two sets of results in Table 4.4 have been noted. The individual PCB congener results for the current work showed a high concentration of PCB No. 28, while Mes *et al.* found none of this congener present in hens eggs samples. The other major difference between the results obtained in the current study and by Mes *et al.* was that, in the current work PCBs No. 138

and 153 were present at higher concentrations than PCBs No. 118 and 180, while in the work of Mes *et al.* all four congeners were present at similar concentrations.

CHAPTER 5: THE DETERMINATION OF PCBS IN COWS MILK SAMPLES

5.1: INTRODUCTION:

The determination of PCBs in cows milk samples has been carried out by a wide range of researchers. The main route of human exposure to many chlorinated organic compounds, including PCBs, is through the ingestion of contaminated food. Cows milk is one of the most important sources of these organic contaminants in the worldwide daily diet, due to its central role in human nutrition. Also, lipophilic organochlorine compounds, such as PCBs, can accumulate in fat-rich milk, and milk products.

Most of the reported methods for the extraction of PCBs from cows milk employed a two-step procedure. The first step involved extracting the PCBs into an organic solvent. Often the milk was mixed with, for example, anhydrous sodium sulphate, and extracted using a Soxhlet apparatus. A number of different clean-up methods have been used including saponification with base, acetonitrile partition, and column chromatography with basic alumina, Florisil, or silica gel. The extracts have been analysed by packed column GC-ECD, capillary column GC-ECD, and capillary column GC-MS. Several of the researchers have analysed the milk extracts for specific individual PCB congeners, and there has been at least one report on the levels of the planar PCB congeners in cows milk.

5.1.1: LEVELS OF PCBS IN COWS MILK:

Tuinstra and Traag (98) analysed the levels of PCBs in Dutch cows milk. The milk samples were centrifuged, and anhydrous sodium sulphate was added to the separated cream. This was extracted by shaking with pentane. The extracts were cleaned up by saponification with alcoholic potassium hydroxide, followed by an alumina column chromatography step. The extracts were analysed by capillary column GC-ECD, and a mixture containing 21 individual PCB congeners

was used to quantify the results. Cows milk samples from three different districts of the Netherlands were analysed. The mean results for 4 of the major individual PCB congeners found in the milk samples were reported for each district. The 4 individual PCB congeners whose results were reported were PCBs No. 52, 101, 138, and 153. PCB No. 52 was found at concentrations between 0.0019 mg/kg and 0.0022 mg/kg on a fat basis in milk samples from the three different districts. Similarly, PCB No. 101 was found at between 0.0025 mg/kg and 0.0035 mg/kg, PCB No. 138 was found at between 0.0070 mg/kg and 0.0135 mg/kg, and PCB No. 153 was found at between 0.0059 mg/kg and 0.0114 mg/kg.

Tuinstra *et al.* (99), again, reported on the levels of PCBs in Dutch cows milk. The extraction and clean-up procedure used was the same as Tuinstra and Traag (98). The samples were analysed by capillary column GC-ECD. A standard solution containing a total of 30 individual PCB congeners was used for quantitation. The levels of six individual PCB congeners (PCBs No. 15, 52, 101, 138, 153, 180) in Dutch milk samples from 1978-1979 were reported. Average levels of between 0.002 mg/kg and 0.014 mg/kg, on a fat basis, were found for the six individual PCB congeners.

A more complete set of results for the content of PCBs in Dutch cows milk was reported by Tuinstra *et al.* (100) in another paper. The fat was isolated from the milk and saponified, and the PCBs were extracted with pentane. The extracts were cleaned up on a basic alumina column, and analysed by capillary column GC-ECD. The extraction and clean-up methods were the same as in Tuinstra and Traag (98). A standard solution containing 30 individual PCB congeners was used for quantitation, just as in Tuinstra *et al.* (99). A total of 165 milk samples were collected from four different areas in the Netherlands. 17 individual PCB congeners were identified in almost all of the milk samples. Nine of the seventeen individual PCB congeners were found to be present at mean values in excess of 0.002 mg/kg fat. The results for the different areas were compared. The different areas included an industrial site and a rural site. Correlation between PCB concentration and the different areas was found only for the hexachlorobiphenyl and heptachlorobiphenyl congeners, and not the lower chlorinated PCBs. The highest levels of individual PCB congeners,

between 0.008 mg/kg and 0.036 mg/kg fat, were found for PCBs No. 15, 138, and 153. The authors estimated a mean Total PCB content of at least 0.083 mg/kg fat in Dutch cows milk.

Steinwandter (101) analysed the levels of PCBs in a sample of cows milk from Germany. The milk sample was mixed with silica gel, and packed into a chromatography column with more silica gel. The PCBs, and organochlorine pesticides, were eluted from the column with an 80:20 (v/v) mixture of light petroleum:dichloromethane. The volume was reduced, and the extracts transferred into hexane ready for analysis by capillary column GC-ECD and GC-MS. A solution containing 9 individual PCB congeners and 26 pesticides was used for quantitation. The individual congeners, PCBs No. 138 and 153 were found to be present at 0.021 mg/kg fat and 0.018 mg/kg fat, respectively.

Heeschen and Blüthgen (102) analysed the levels of PCBs in German cows milk samples. Six individual PCB congeners were analysed, PCBs No. 28, 52, 101, 138, 153, and 180. No extraction methodology was reported. A total of 911 cows milk samples were analysed for the six individual congeners, and for Total PCBs using Clophen A60 as the quantitation standard. The samples were found to contain average concentrations of the six congeners of between 0.001 mg/kg fat and 0.014 mg/kg fat, and an average Total PCB concentration of 0.111 mg/kg fat. A total of 363 cows milk samples collected from tankers were also analysed. These were found to contain the six congeners at average concentrations between 0.0009 mg/kg fat and 0.013 mg/kg fat, and an average Total PCB concentration of 0.102 mg/kg fat. The values obtained for the six congeners and for Total PCBs were compared with values from different European countries. All of the milk samples from the various countries were found to contain similar Total PCB concentrations, and similar concentrations of the six individual congeners. In addition, a Total PCB concentration of 3 mg/kg fat was reported for German human milk samples.

Pines *et al.* (103) studied the levels of PCBs in 42 samples of farm cows milk and 41 samples of market milk from Israel. The samples were taken in three separate batches, 10 farm cows milk and 10 market milk samples in 1976, 14 farm cows milk and 16 market milk samples in 1983, and 18 farm cows milk and 15 market milk samples in 1986. The milk samples were

extracted using a Florisil column chromatography step. The PCBs were then separated from the other organochlorine compounds by a silicic acid column chromatography step. The extracts were analysed by packed column GC-ECD, and Aroclor 1254 was used to quantify the tetra-, penta-, and hexachlorobiphenyls. The Total PCB concentrations found were markedly lower for the samples collected in 1986, than those collected in 1976 or 1983. The Total PCB concentrations in 1976 were 0.011 mg/kg in farm cows milk and 0.010 mg/kg in market milk. In 1983 the PCB concentration in farm cows milk samples was roughly the same at 0.012 mg/kg, while the PCB concentration in the market milk samples was lower at 0.005 mg/kg. Bcth sets of milk samples showed lower PCB concentrations in 1986, with the farm cows milk at 0.001 mg/kg and the market milk at 0.001 mg/kg also. All of the results were reported on a whole milk basis.

Frank and Braun (104) analysed the levels of PCBs in 1,184 milk samples from Ontario, Canada in 1985-1986. The milk samples were collected from bulk transporters hauling milk from all areas of Ontario. The milk samples were extracted with hexane using a Soxhlet apparatus, and cleaned up by Florisil column chromatography. The extracts were fractionated by charcoal column chromatography. The PCBs were analysed by packed column GC-ECD, and Aroclors 1254 and 1260 were used for quantitation. The Total PCB results for the 1,184 milk samples were split into results for five different regions of Ontario. The mean overall Total PCB concentration found for the 1,184 milk samples was 0.015 mg/kg, with the regional mean values varying from 0.012 mg/kg to 0.019 mg/kg. 95 % of the 1,184 milk samples contained PCBs at a concentration of between 0.005 mg/kg and 0.050 mg/kg. The Total PCB concentration in milk samples from the Southern region of Ontario were found to have fallen between 1970 and 1986 from 0.085 mg/kg to 0.019 mg/kg. All of the results were reported on a fat basis.

McLachlan (105) investigated the levels of PCBs in the milk of a single cow, and compared this to the levels found in the cattle feed. The milk was collected daily and centrifuged, the fat layer was skimmed off, and the samples frozen until extraction. The milk was extracted by mixing with anhydrous sodium sulphate, and packing into a column. The sample was extracted with a mixture of acetone:hexane (1:2). The extracts were cleaned up using three separate steps. A gel

permeation chromatography step was followed by a Florisil column chromatography step and, finally, an alumina column chromatography step. The extracts were analysed by capillary column GC-MS, and the PCBs were quantified using a 1:1:1:1 mixture of Clophens A30, A40, A50, and A60. The PCBs were quantified by homologue for tri- to decachlorobiphenyls. The total amount of PCBs found in the collected milk was 15,000 ng/day. The cow was found to produce approximately 1.4 kg of milkfat per day, which gave a mean Total PCB concentration of 10.7 ng/g fat.

Frank *et al.* (96) analysed the levels of PCBs in the general diet in Ontario, Canada from 1986-1987. The milk samples were extracted with hexane using a Soxhlet apparatus, and cleaned up by Florisil column chromatography. The PCBs were fractionated from co-extractants by charcoal column chromatography. The extracts were analysed by packed column GC-ECD, and Aroclors 1254 and 1260 were used for quantitation. The mean Total PCB concentration in the Canadian cows milk samples was found to be 6×10^{-4} mg/kg on a whole milk basis.

Mes *et al.* (43) determined the levels of 34 specific PCB isomers in a variety of foodstuffs, including cows milk, in the Canadian diet. The milk samples were homogenised with a 1:1 (v/v) mixture of acetone:hexane, and centrifuged. The upper phase was filtered through a column containing anhydrous sodium sulphate. The PCBs were separated from the lipid material by gel permeation chromatography, and cleaned up by Florisil column chromatography. The extracts were saponified with methanolic potassium hydroxide, and collected in hexane. The PCBs were analysed and quantified by capillary column GC-ECD. Confirmation of peak identities was done by capillary column GC-MS. A solution containing the 34 individual PCB congeners of interest was used to quantify the extracts. The Total PCB concentration was found to be 2×10^{-4} mg/kg in whole milk, and 1×10^{-4} mg/kg in 2 % milk on a wet weight basis. The individual congeners PCBs No. 28, 52, 66, 118, 138, 153, and 180 were found in the two different types of milk, at concentrations between 11 ng/kg and 44 ng/kg wet weight.

Brunn *et al.* (97) analysed PCBs in a range of foodstuffs, including cows milk, from Germany. 35 cows milk samples were analysed. The samples were Soxhlet extracted, and the

PCBs were separated from the co-extracted fat by an alumina column chromatography step. The extracts were analysed for 7 individual PCB congeners, PCBs No. 28, 49, 52, 101, 138, 153, and 180, by capillary column GC. Each sample was analysed twice on two different columns. Only two of the 7 individual congeners, PCBs No. 138 and 153, were found in the cows milk samples. The two PCB congeners were present at average concentrations of 0.018 mg/kg fat and 0.033 mg/kg fat for PCBs No. 138 and 153, respectively. The average Total PCB concentration for the 7 individual congeners was 0.051 mg/kg fat.

Van Rhijn *et al.* (106) analysed the levels of planar PCB congeners in Dutch cows milk. The milk samples were extracted by liquid-liquid extraction. The extracts were cleaned up by gel permeation chromatography, followed by alumina column chromatography. A porous graphitised carbon (PGC) column chromatography step was used to separate the planar PCB congeners, as well as the PCDDs and PCDFs, from the other PCB congeners. The extracts were analysed by capillary column GC-MS. A standard solution containing the three PCB congeners of interest, PCBs No. 77, 126, and 169, was used for quantitation. The 39 milk samples that were analysed were taken from 9 different sites, representing 4 different types of grazing areas. All three of the planar PCB congeners were found in all of the samples. PCB No. 77 was found at concentrations between 2.8 ng/kg and 11.9 ng/kg. PCB No. 126 was found at concentrations between 11.6 ng/kg and 42.9 ng/kg. PCB No. 169 was found at concentrations between 2.0 ng/kg and 10.2 ng/kg. All of the results were reported on a fat basis. The milk samples taken from agricultural areas were found to contain the lowest concentrations of the planar PCB congeners, whereas the milk samples taken from the surroundings of municipal waste incinerators had the highest concentrations.

5.2: RESULTS:

5.2.1: RESULTS FOR UEA COWS MILK SAMPLES:

A total of 8 cows milk samples were analysed for 7 individual PCB congeners by capillary column GC-MS, as outlined in Chapter 2. The results for the 7 individual PCB congeners in the 8 cows milk samples have been tabulated. Table 5.1 shows the PCB concentration results for the 8 cows milk samples quantified by the ICES method outlined in Section 2.3.1.1. The Total PCB concentrations for the 8 cows milk samples, calculated by summing the 7 individual congener concentrations and multiplying by a factor of four, ranged from 6.8 μg/l whole milk to 45.2 μg/l whole milk. The mean Total PCB concentration for the 8 cows milk samples, as quantified by the ICES method, was 16.8 μg/l whole milk.

PCB No.	Concentration of PCB Congeners in μg/l by ICES Method							
	COWS MILK SAMPLES							
	RA	RD	RG	RJ	RK	RL	RM	RN
28	1.5	0.4	0.6	0.6	1.2	0.3	0.4	0.9
52	1.3	0.4	0.6	0.4	0.8	0.1	0.3	0.6
101	0.8	0.3	0.3	0.3	0.6	0.1	0.1	0.2
118	0.3	0.2	0.1	0.1	0.7	0.4	0.1	0.2
138	1.1	0.4	0.3	0.3	3.0	0.9	0.3	0.5
153	1.0	0.5	0.4	0.3	2.9	1.0	0.3	0.4
180	0.6	0.4	0.2	0.1	2.1	0.4	0.2	1.0
Total PCBs	26.4	10.4	10.0	8.4	45.2	12.8	6.8	15.2

TABLE 5.1: CONCENTRATION OF PCBS IN COWS MILK SAMPLES BY THE ICES METHOD

The results shown in Table 5.1 were the average of duplicate analyses for each cows milk extract. All of the results had been corrected for the experimental percentage recoveries, which were calculated as outlined in Section 2.3.1.1. The experimental percentage recoveries for the 8 cows milk samples ranged from 74.7 % to 84.0 %. The two blank extracts (Section 2.3.1.1) did not show peaks at the correct experimental retention times for any of the 7 individual PCB congeners.

The 8 cows milk samples were re-extracted, and the results, again as calculated by the ICES method, are shown in Table 5.2. This time the results were lower than the first extractions, with the Total PCB concentrations found ranging from 2.4 μg/l whole milk to 8.0 μg/l whole milk. The mean Total PCB concentration for the 8 re-extracted cows milk samples was 5.2 μg/l whole milk.

PCB No.	Concentration of PCB Congeners in μg/l by ICES Method							
	COWS MILK SAMPLES							
	RA	RD	RG	RJ	RK	RL	RM	RN
28	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.8
52	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.4
101	0.1	0.1	ND	0.1	0.2	0.1	0.2	0.3
118	0.1	0.1	ND	0.1	0.2	0.1	0.1	0.1
138	0.2	0.3	0.1	0.1	0.3	0.4	0.2	0.2
153	0.2	0.2	0.1	0.1	0.4	0.4	0.2	0.2
180	0.2	0.2	0.1	ND	0.3	0.3	ND	ND
Total PCBs	4.4	4.8	2.4	2.4	6.8	6.8	4.4	8.0

TABLE 5.2: CONCENTRATION OF PCBS IN RE-EXTRACTED COWS MILK SAMPLES BY THE ICES METHOD

The mean concentrations for the 7 individual PCB congeners for both extractions are shown in Table 5.3, and are displayed in Diagram 5.1.

PCB No.	Mean Concentration of PCB Congeners in µg/l Milk	
	1st Extr.	2nd Extr.
28	0.74	0.26
52	0.56	0.16
101	0.34	0.14
118	0.26	0.1
138	0.85	0.23
153	0.85	0.23
180	0.63	0.14

TABLE 5.3: MEAN CONCENTRATIONS OF INDIVIDUAL PCB CONGENERS IN 2 EXTRACTIONS OF COWS MILK SAMPLES

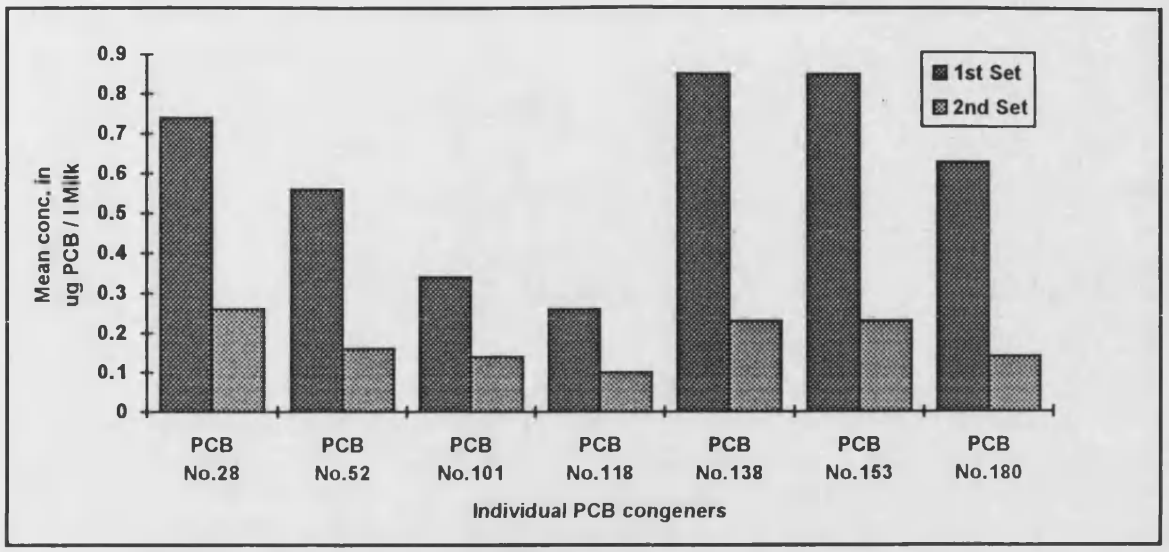


DIAGRAM 5.1: MEAN CONCENTRATIONS OF INDIVIDUAL PCB CONGENERS IN COWS MILK SAMPLES

5.2.2: RESULTS FOR MAFF COWS MILK SAMPLES:

5.2.2.1: Original Results:

A number of cows milk samples collected from two different farms were analysed, as outlined in Chapter 2. The cows milk samples were analysed by capillary column GC-MS, and the PCB concentrations were calculated by homologue. The results originally obtained for the concentrations of PCBs, by chlorination level, in the cows milk samples from the two farms are shown in Tables 5.4 and 5.5. The results for the cows milk samples collected from J. & G. Jackson, Teuthill Farm, Alvanley are labelled "Jackson cows milk samples" in the Tables, while those collected from J. B. Allwood & Sons, Milton Brook Lodge, Great Barrow, Cheshire are labelled "Control cows milk samples".

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK								
	BATCH No.								
	1	2(i)	2(ii)	3(i)	3(ii)	4(i)	4(ii)	5(i)	5(ii)
2Cl	ND	0.0005	0.0006	ND	ND	ND	ND	ND	0.0016
3Cl	ND	0.0117	0.0105	0.0080	0.0040	0.0020	0.0024	0.0018	0.0032
4Cl	0.0030	0.0031	0.0015	ND	ND	0.0025	0.0008	0.0037	0.0019
5Cl	0.0005	ND	0.0009	0.0040	ND	ND	0.0008	ND	ND
6Cl	0.0025	ND	ND	ND	ND	ND	ND	ND	ND
7Cl	ND	ND	ND	ND	ND	ND	ND	ND	ND
TOTAL	0.0060	0.0153	0.0135	0.0120	0.0040	0.0045	0.0040	0.0055	0.0067

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK								
	BATCH No.								
	6(i)	6(ii)	7	8(i)	8(ii)	9(i)	9(ii)	10(i)	10(ii)
2Cl	0.0005	0.0007	ND	0.0012	0.0006	0.0003	0.0010	0.0005	0.0006
3Cl	0.0031	0.0026	0.0029	0.0040	0.0026	0.0062	0.0041	0.0046	0.0008
4Cl	0.0017	0.0085	0.0015	0.0032	0.0017	0.0039	0.0011	0.0008	0.0017
5Cl	0.0011	ND	ND	0.0003	0.0014	0.0019	0.0001	0.0001	ND
6Cl	0.0003	ND	ND	ND	ND	0.0005	0.0002	ND	ND
7Cl	ND	0.0008	ND	ND	ND	ND	ND	ND	0.0012
TOTAL	0.0068	0.0126	0.0045	0.0086	0.0062	0.0129	0.0065	0.0060	0.0043

N.B. (i) & (ii) refer to duplicate extractions of the same milk sample

TABLE 5.4: COMPOSITION OF PCBS IN JACKSON COWS MILK SAMPLES

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK							
	BATCH No.							
	2(i)	2(ii)	3	4(i)	4(ii)	5	6(i)	6(ii)
2Cl	ND	ND	ND	0.0006	ND	0.0011	0.0014	ND
3Cl	0.0020	ND	ND	0.0035	0.0011	0.0046	0.0065	0.0070
4Cl	0.0015	0.0020	0.0016	0.0004	0.0036	0.0012	0.0028	0.0045
5Cl	0.0013	0.0016	0.0022	ND	0.0017	0.0009	ND	0.0007
6Cl	ND	0.0007	ND	ND	0.0007	0.0002	ND	ND
7Cl	ND	0.0013	ND	ND	0.0009	ND	ND	ND
TOTAL	0.0048	0.0056	0.0038	0.0046	0.0079	0.0080	0.0106	0.0122

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK							
	BATCH No.							
	7(i)	7(ii)	8(i)	8(ii)	9(i)	9(ii)	10(i)	10(ii)
2Cl	0.0011	0.0009	ND	ND	0.0004	ND	0.0001	0.0018
3Cl	0.0033	0.0051	0.0053	0.0039	0.0025	0.0008	0.0008	0.0024
4Cl	0.0026	0.0015	ND	0.0006	0.0027	0.0008	0.0014	0.0030
5Cl	ND	ND	ND	0.0003	0.0007	0.0003	0.0002	ND
6Cl	ND	ND	ND	ND	0.0002	0.0001	0.0001	ND
7Cl	ND	ND	ND	ND	ND	ND	ND	ND
TOTAL	0.0070	0.0074	0.0053	0.0049	0.0065	0.0020	0.0026	0.0072

N.B. (i) & (ii) refer to duplicate extractions of the same milk sample

TABLE 5.5: COMPOSITION OF PCBS IN CONTROL COWS MILK SAMPLES

The results for the milk samples from the two different farms were compared, to find out whether the Jackson samples contained higher PCB concentrations than the Control samples. A breakdown of the mean PCB concentrations by level of chlorination is shown in Table 5.6.

LEVEL OF CHLORINATION	JACKSON COWS MILK SAMPLES (n=18)		CONTROL COWS MILK SAMPLES (n=16)	
	MEAN CONC. (mg/kg Whole Milk)	% w/w	MEAN CONC. (mg/kg Whole Milk)	% w/w
2Cl	0.0005	6.4	0.0005	7.9
3Cl	0.0041	52.6	0.0031	49.2
4Cl	0.0023	29.5	0.0019	30.2
5Cl	0.0006	7.7	0.0006	9.5
6Cl	0.0002	2.5	0.0001	1.6
7Cl	0.0001	1.3	0.0001	1.6
TOTAL	0.0078		0.0063	

TABLE 5.6: COMPARISON OF MEAN PCB COMPOSITIONS BY HOMOLOGUE IN JACKSON AND CONTROL COWS MILK SAMPLES

The mean results for the two farms were very similar, as Table 5.6 clearly shows. The results from Table 5.6 are displayed in Diagram 5.2.

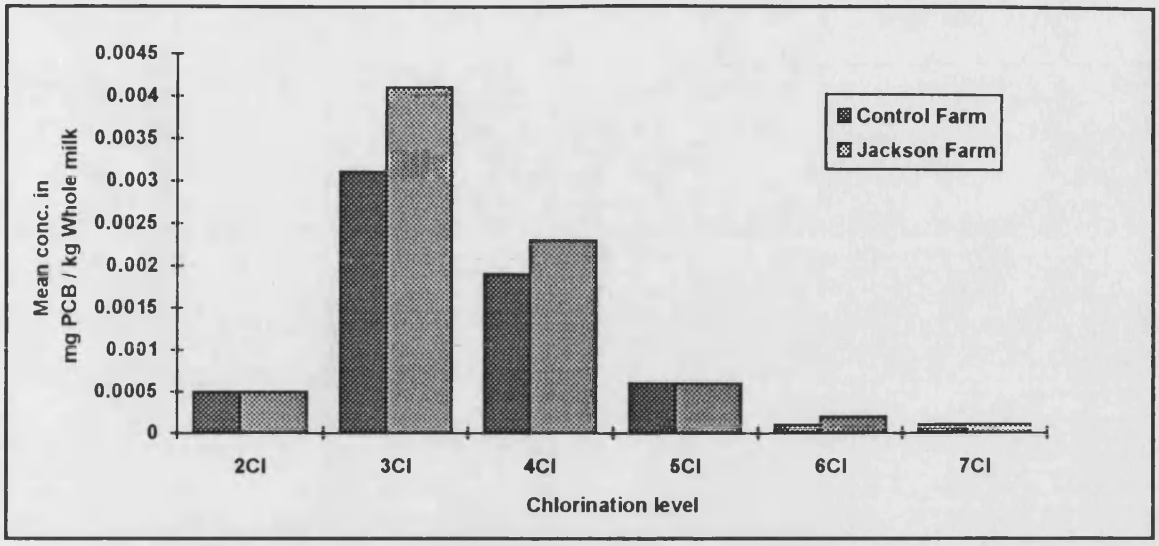


DIAGRAM 5.2: COMPARISON OF PCB CONCENTRATIONS IN COWS MILK SAMPLES FROM TWO DIFFERENT FARMS

Diagram 5.2 also shows that most of the PCBs found in the cows milk samples from both farms were trichloro- and tetrachlorobiphenyls. 82 % of the Total PCBs in the Jackson cows milk samples, and 79 % of the Total PCBs in the Control cows milk samples were trichloro- and tetrachlorobiphenyls. The patterns of the PCBs found by chlorination level in the cows milk samples closely resembled the PCB pattern found in Aroclor 1242, Diagram 5.3. This resemblance suggested that the source that was responsible for contaminating the cows milk must have used or contained Aroclor 1242.

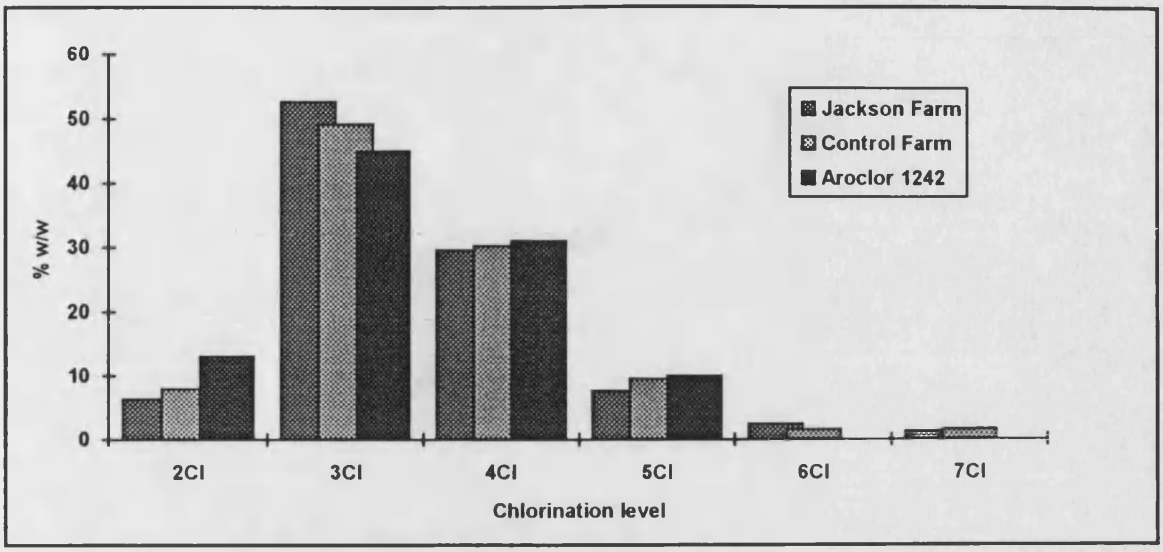


DIAGRAM 5.3: COMPARISON OF PCBS IN COWS MILK SAMPLES BY CHLORINATION LEVEL WITH AROCLOR 1242

The problem with the results reported here was the rather wide discrepancies in PCB concentrations found in duplicate extracts from the same cows milk sample, i.e. two aliquots taken from the same bottle of cows milk, and extracted and analysed separately.

A series of discussions with representatives of MAFF led to several changes in the analytical protocol being made, which were designed to improve the reproducability of the PCB results.

Firstly, the HPLC column was cleaned more regularly between runs, or batches of runs. Stronger solvents such as pure heptane or heptane:THF were used, and the time taken for washing was increased. This was designed to minimise the carry-over of PCB residues from one extract to another. Any carry-over between extracts would adversely affect the accuracy of the final PCB result.

Secondly, it was decided to use a five figure digital balance to determine exactly the final volumes of the extracts. This would give a more accurate measurement of the final volume than simply using a graduated syringe to adjust the volume to 1 ml. It would also allow a correction to be made in the calculation of the PCB concentration present for variations in the final volume

above or below the assumed value of 1 ml. This step would, therefore, improve the accuracy of the final PCB concentrations reported.

Finally, visual inspection of the GC-MS traces, as well as mathematical testing, was used to determine which peaks were due to PCBs and which were not. When looking for very low levels of PCBs, near to the limit of quantitation of the GC-MS, rigidly sticking to the rule of ratioing areas for two masses and checking against recorded values can lead to the exclusion of some peaks which were probably due to PCBs, and less commonly the inclusion of spurious peaks. At such low levels, the failure to include or exclude just one peak on a chromatogram can make a relatively large difference to the final PCB concentration reported. Visual inspection, in tandem with mathematical testing, was expected to show a marked improvement in the reproducability of the reported results.

5.2.2.2: Re-Analysed Results:

The use of visual inspection outlined above was put into practice, and a number of the milk samples were re-analysed. The recalculated results are shown in Table 5.7 for 4 samples of Jackson cows milk.

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK							
	BATCH No.							
	3(i) a.	3(i) b.	Mean	%w/w	3(ii) a.	3(ii) b.	Mean	%w/w
2Cl	0.0015	0.0015	0.0015	15.8	0.0011	0.0011	0.0011	15.5
3Cl	0.0038	0.0040	0.0039	41.1	0.0032	0.0032	0.0032	45.1
4Cl	0.0030	0.0028	0.0029	30.5	0.0020	0.0020	0.0020	28.2
5Cl	0.0013	0.0013	0.0013	13.7	0.0009	0.0009	0.0009	12.7
6Cl	ND	ND	ND	0	ND	ND	ND	0
TOTAL	0.0095	0.0095	0.0095		0.0071	0.0071	0.0071	
	6(i) a.	6(i) b.	Mean	%w/w	6(ii) a.	6(ii) b.	Mean	%w/w
2Cl	0.0009	0.0011	0.0010	12.2	0.0012	0.0012	0.0012	11.7
3Cl	0.0029	0.0030	0.0030	36.6	0.0038	0.0040	0.0039	37.9
4Cl	0.0024	0.0021	0.0023	28.0	0.0026	0.0030	0.0028	27.2
5Cl	0.0011	0.0011	0.0011	13.4	0.0015	0.0013	0.0014	13.6
6Cl	0.0009	0.0009	0.0009	11.0	0.0010	0.0010	0.0010	9.7
TOTAL	0.0082	0.0082	0.0082		0.0101	0.0104	0.0103	
	8(i) a.	8(i) b.	Mean	%w/w	8(ii) a.	8(ii) b.	Mean	%w/w
2Cl	0.0006	0.0006	0.0006	6.1	0.0003	0.0004	0.0004	5.9
3Cl	0.0038	0.0036	0.0037	37.4	0.0023	0.0026	0.0025	36.8
4Cl	0.0040	0.0040	0.0040	40.4	0.0020	0.0029	0.0025	36.8
5Cl	0.0008	0.0011	0.0010	10.1	0.0009	0.0009	0.0009	13.2
6Cl	0.0004	0.0006	0.0005	5.1	0.0006	0.0006	0.0006	8.8
TOTAL	0.0098	0.0100	0.0099		0.0061	0.0074	0.0068	

	9(i) a.	9(i) b.	Mean	%w/w	9(ii) a.	9(ii) b.	Mean	%w/w
2Cl	0.0011	0.0011	0.0011	12.1	0.0008	0.0008	0.0008	10.8
3Cl	0.0032	0.0034	0.0033	36.3	0.0025	0.0025	0.0025	33.8
4Cl	0.0029	0.0027	0.0028	30.8	0.0023	0.0022	0.0023	31.1
5Cl	0.0011	0.0011	0.0011	12.1	0.0010	0.0010	0.0010	13.5
6Cl	0.0009	0.0009	0.0009	9.7	0.0008	0.0008	0.0008	10.8
TOTAL	0.0091	0.0091	0.0091		0.0075	0.0074	0.0074	

(i) & (ii) refer to duplicate extractions of the same milk sample
a. & b. refer to duplicate analyses of the same milk extract

TABLE 5.7: RE-ANALYSIS OF SELECTED JACKSON COWS MILK SAMPLES

A direct comparison between the original and re-analysed results for Total PCBs for these four samples are shown in Table 5.8.

SAMPLE No.	CONC. IN mg/kg WHOLE MILK	
	ORIGINAL RESULT	RE-ANALYSED RESULT
3(i)	0.0120	0.0095
3(ii)	0.0040	0.0071
6(i)	0.0068	0.0082
6(ii)	0.0126	0.0103
8(i)	0.0086	0.0099
8(ii)	0.0062	0.0068
9(i)	0.0129	0.0091
9(ii)	0.0065	0.0074
11(i)	0.0054	0.0097
11(ii)	0.0103	0.0130
12(i)	0.0140	0.0147
12(ii)	0.0107	0.0153

TABLE 5.8: SUMMARY OF MEAN PCB RESULTS FOR SELECTED ORIGINAL AND RE-ANALYSED JACKSON COWS MILK SAMPLES

In addition, two new Jackson milk samples were extracted and analysed by the two different methods, and these results have also been compared in Table 5.8. Table 5.8 shows the improvement in the agreement between duplicate results in most of the samples upon the adoption of the use of visual inspection, along with mathematical testing of peaks.

The precision of the analysis of PCBs by GC-MS was tested using both the original and the new method of peak identification. The precision results for the original method are shown in Table 5.9, while those for the visual inspection method are shown in Table 5.10.

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg ($x10^4$) WHOLE MILK		
	MEAN	STANDARD DEVIATION	RELATIVE STANDARD DEVIATION
2Cl	11.1	+/-1.66	+/-14.9%
3Cl	30.5	+/-7.43	+/-24.4%
4Cl	15.6	+/-5.83	+/-37.4%
5Cl	5.8	+/-3.00	+/-51.7%
TOTAL	63.8	+/-11.11	+/-17.6%

TABLE 5.9: TEST OF GC-MS PRECISION - One extract of cows milk analysed ten times

LEVEL OF CHLORINATION	PCB CONCENTRATION IN mg/kg WHOLE MILK					
	1	2	3	4	5	6
2Cl	0.0012	0.0012	0.0013	0.0012	0.0012	0.0012
3Cl	0.0038	0.0040	0.0041	0.0040	0.0040	0.0040
4Cl	0.0026	0.0030	0.0026	0.0026	0.0025	0.0026
5Cl	0.0015	0.0013	0.0013	0.0013	0.0013	0.0013
6Cl	0.0010	0.0010	0.0010	0.0010	0.0010	0.0012
TOTAL	0.0101	0.0104	0.0104	0.0101	0.0099	0.0103

TABLE 5.10: TEST OF GC-MS PRECISION - One extract of cows milk analysed six times

TOTAL PCBs: Mean (x) = 0.0102 mg/kg Whole Milk
Standard Deviation (s) = 0.0002
No. of Samples (n) = 6
Relative Standard Deviation (RSD) = 2.0 %

The improvement in the precision achieved by switching to the visual inspection method can be seen from the decrease in the relative standard deviation (RSD) of the Total PCB result for the new method. The RSD for the original method was 17.6 %, while the RSD for the visual inspection method was only 2.0 %. It can also be seen from Tables 5.9 and 5.10 that the improvement in precision was greater for the homologues with lower actual PCB concentrations than for those with higher concentrations. This supported the statement that visual inspection was needed to achieve a greater degree of reproducability for low concentrations of PCBs, close to the limit of quantitation.

5.2.2.3: Individual PCB Congener Results:

A number of individual PCB congeners were identified in 5 of the Jackson cows milk samples, using both the MS pattern of an individual peak as well as the GC retention time. The

individual PCB congeners under investigation included the eleven PCBs present in RPCBR-1 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), PCBs No. 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, 209. A standard solution, called PCB Mix No. 3, containing PCBs No. 28, 52, 101, 118, 138, 153, and 180, was also used (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.). A mixture of 12 individual congeners called SS1010, containing PCBs No. 18, 28, 52, 74, 101, 138, 153, 156, 180, 189, 194, and 206 was also analysed. This solution was sent from Canada. Finally, a mixture of the 4 planar PCB congeners, PCBs No. 77, 80, 126, 169, was prepared from solutions of the individual congeners, which were purchased from Ultrascientific Inc., Hope, U.S.A.

The individual PCB congeners positively identified in the 5 samples are shown in Table 5.11. A number of these individual PCB congeners, e.g. PCBs No. 52, 101, 138, 153, have been found by many previous researchers to be present in a range of foodstuffs, and other samples.

BATCH No.	INDIVIDUAL PCB CONGENERS IDENTIFIED
8(i)	18, 28, 52, 74, 101, 138, 153
8(ii)	18, 52, 74, 101, 138, 153
11(ii)	18, 28, 52, 74, 97, 101, 118, 138, 153, 180
12(i)	18, 28, 52, 74, 77/80, 101, 118, 138, 153
12(ii)	18, 28, 52, 74, 101, 118, 138, 153
PCB CONGENERS INVESTIGATED	1, 3, 7, 18, 28, 30, 50, 52, 74, 77/80, 97, 101, 118, 126, 138, 143, 153, 156, 169, 180, 183, 189, 194, 202, 206, 207, 209

TABLE 5.11: INDIVIDUAL PCB CONGENERS IDENTIFIED IN JACKSON COWS MILK SAMPLES BY GC-MS

5.3: DISCUSSION:

5.3.1: DISCUSSION FOR UEA COWS MILK SAMPLES:

The mean Total PCB concentration found in the 8 cows milk samples are compared with other previous results in Table 5.12.

REF No.	No. of Samples	Mean Total PCB Concentration in ppb Whole Milk
103. Pines *et al.*	10, 14, 18	11, 11.5, 1
103. Pines *et al.*	10, 16, 15	10, 5, 1
43. Mes *et al.*	NA	0.2
96. Frank *et al.*	NA	0.6
Current Work	8	5.2

TABLE 5.12: COMPARISON OF MEAN TOTAL PCB CONCENTRATIONS FOUND IN COWS MILK SAMPLES

The mean Total PCB concentration of 5.2 ppb whole milk was higher than those found by Mes *et al.* (43) and Frank *et al.* (96), but in agreement with the results of Pines *et al.* (103). All of the researchers used different quantitation standards. Mes *et al.* (43) found a Total PCB concentration of 0.2 ppb whole milk, and used a set of 34 individual PCB congeners for quantitation. Frank *et al.* (96) found a Total PCB concentration of 0.6 ppb whole milk, and used a mixture of Aroclors 1254 and 1260 for quantitation. Pines *et al.* (103) found mean Total PCB concentrations of between 1 ppb whole milk and 11.5 ppb whole milk, and used Aroclor 1254 as the quantitation standard.

The mean concentrations for the 7 individual PCB congeners have been compared with the results of Mes *et al.* (43) in Table 5.13.

REF No.	No. of Samples	Concentration of PCB Congeners in ppb Whole Milk						
		PCB Congener No.						
		28	52	101	118	138	153	180
43. Mes *et al.*	NA	0.011	0.053	ND	0.044	0.024	0.027	0.019
Current Work	8	0.26	0.16	0.14	0.10	0.23	0.23	0.14

TABLE 5.13: COMPARISON OF INDIVIDUAL PCB CONGENERS FOUND IN COWS MILK SAMPLES

The actual concentrations of the 7 individual PCB congeners were higher in the current work than found by Mes *et al.* In addition, the current work found relatively high concentrations of PCB No. 28, while Mes *et al.* found only a low concentration of this congener. In the current work, PCBs No. 138 and 153 were found at higher concentrations than PCBs No. 118 and 180, while Mes *et al.* found a higher concentration of PCB No. 118 with PCBs No. 138, 153, and 180 all present at roughly the same concentration. Mes *et al.* did not determine PCB No. 101 in the cows milk samples.

The Total PCB concentrations found in the 8 cows milk samples were slightly higher than some of those previously reported. The results for the 7 individual PCB congeners were, also, slightly higher than found by previous researchers. However, no more conclusions could be drawn from the individual PCB congener results obtained for the 8 cows milk samples.

5.3.2: DISCUSSION FOR MAFF COWS MILK SAMPLES:

Three main points can be drawn from the cows milk results presented here. Firstly, the mean Total PCB concentrations found in the Jackson cows milk samples (0.0078 mg/kg Whole Milk) and the Control cows milk samples (0.0063 mg/kg Whole Milk) were similar. Secondly, the PCB

congeners that were positively identified in the two sets of cows milk samples were similar in their distributions and concentrations by level of chlorination. Lastly, the distribution by chlorination level of the PCB congeners in the two sets of cows milk samples matched the composition of Aroclor 1242 more closely than any other Aroclor.

The mean Total PCB concentrations found in the two sets of samples were similar to the highest levels found in British cows milk samples by MAFF between 1967 and 1978 (86). A total of 253 samples were analysed in the MAFF report, and only 4 samples were found to have had a mean Total PCB concentration of 0.006 mg/kg. In addition, 5 samples had a mean Total PCB concentration of 0.004 mg/kg, 1 sample had a Total PCB concentration of 0.002 mg/kg, and 6 samples had a mean Total PCB concentration of < 0.002 mg/kg. PCBs were not detected in 101 of the samples, and mean values were not reported for 136 samples.

One of the most important aspects of the results reported here was the presence of quantifiable levels of PCBs in all of the cows milk samples that were analysed from both farms. The mean Total PCB concentrations found at the two farms were similar, which suggested that the contamination of cows milk was not simply confined to farms very near to the disused landfill site. This landfill site had been suspected as the source of the PCB contamination of the Jackson cows milk. If this assumption was correct, then the contamination of cows milk was more widespread than had been originally thought. Alternatively, other sources of PCBs may have caused the contamination found in this work. No change in the Total PCB concentrations for either farm was seen during the twelve weeks of the study, suggesting that the environmental pollution in this area was stable and long term. Small variations in the PCB levels found each week are to be expected, some of which are caused by differences in the height of the grass, and the exact location for the collection of the grass samples.

The results reported here emphasise the need to monitor regularly the concentrations of PCBs to be found in cows milk samples from around the country. The importance of cows milk in the daily diet of the population means that much more information needs to be gathered about the precise levels of PCBs, and other pollutants, present in milk.

CHAPTER 6: THE DETERMINATION OF PCBS IN HUMAN BREAST MILK SAMPLES

6.1: INTRODUCTION:

The analysis of PCBs in human milk has been carried out by a range of researchers. Long-term, low-level exposure of women to PCBs in the diet and the environment results in a gradual accumulation of PCBs in the fat, including the fat of breast milk. Lactation is the most important way in which large amounts of such PCB residues can be eliminated by mothers. The presence of PCB residues in breast milk has been carefully monitored for two main reasons. Firstly, because of the possibility of fairly high levels of PCBs accumulating in the breast milk. The stability and lipophilicity of the PCBs, and the position of humans at the top of the food chain are responsible for the possibility of high PCB levels being present in the breast milk. Secondly, because of concern for the developing infant, who may be most vulnerable to these chemical contaminants. The infant is poorly equipped to deal with the possible toxic effects of PCBs.

Comparison of PCB concentrations reported in breast milk samples from different studies must be done with caution. This is because the fat content of breast milk varies widely from woman to woman, and increases over the course of a feeding. The PCB concentrations will be reported either on a fat basis or a whole milk basis, and this must be clearly stated for comparisons to be made, because the concentrations in fat will usually be about 30 times higher than the concentrations in whole milk (107).

A number of investigations have confirmed that breast feeding is the major transfer route of PCBs from mother to baby. Other routes such as placental transfer have been found to be minor. In general, a 30-50 % decrease in the level of PCBs in the mother's tissues or fluids has been observed after 5 to 6 months of breast feeding. This 30-50 % of PCBs has been transferred from the mother to the infant (108).

6.1.1: LEVELS OF PCBS IN HUMAN BREAST MILK:

Some of the early results for the levels of PCBs found in human milk samples from around the world are presented in Tables 6.1-6.3 below. Some of these results are discussed more fully in this section, along with other more recent work on the levels of PCBs in human milk.

COUNTRY OF ORIGIN	SAMPLING YEAR	NO. OF SUBJECTS	MEAN PCB LEVEL (ng/g)
Australia	1971-72	45	0
Canada	1974	15	19.6
Canada	1975	100	12
Germany	NK	NK	103
Germany	1970	NK	3.0
Germany	1973	96	90
Israel	1975	28	44.2
Japan	1972	31	23.8
Japan	1972	4	22.8
Japan	1972-73	402	28.2
Japan	1972-74	NK	40.0
Japan	1972-76	341	25.0
New Guinea	1970	32	0
Norway	1969-70	42	11.0
Norway	1976	44	24.1
Sweden	1967	NK	14
Sweden	1971-72	NK	26
Sweden	1974	NK	24
Sweden	1976-77	NK	30
U.S.A.	1970	47	0
U.S.A.	1971-72	39	9.8
U.S.A.	NK	1,038	45.0

NK = not known.

TABLE 6.1: TOTAL PCB CONCENTRATIONS IN WHOLE BREAST MILK FROM AROUND THE WORLD, 1967-1977

From Landrigan in Kimbrough (3), pg. 270.

COUNTRY OF ORIGIN	SAMPLING YEAR	NO. OF SUBJECTS	MEAN PCB LEVEL (ng/g)
Austria (urban)	NK	NK	1,540
Austria (rural)	NK	NK	1,290
Canada	1969-74	96	1,100
Canada	1974	15	1,728
Germany	1971	NK	3,500
Japan	1972	31	768
Japan	1972	314	1,200
Japan	1972-76	341	955
Poland	NK	179	359
Sweden	1967	NK	460
Sweden	1971-72	NK	870
Sweden	1974	NK	810
Sweden	1976-77	NK	930

NK = not known

TABLE 6.2: TOTAL PCB CONCENTRATIONS IN BREAST MILK FAT FROM AROUND THE WORLD, 1967-1977

From Landrigan in Kimbrough (3), pg. 271.

COUNTRY OF ORIGIN	SAMPLING YEAR	MEAN PCB LEVEL (ng/g)
Japan	1977	1,100
U.S.A. (mainland)	1977-78	1,500
U.S.A. (Hawaii)	1979-80	800
Canada (native pop.)	NK	530
Canada (national)	NK	420
Great Britain	1979-80	500
Germany	1979-81	1,800
Norway	1981-82	1,000
Denmark	1982	800
Sweden	1979	1,400
Belgium	NK	600
Finland	1984-85	930
Yugoslavia	NK	500
Israel	NK	540
Thailand	NK	60
Vietnam	NK	100
India	1988	120

NK = not known

TABLE 6.3: TOTAL PCB CONCENTRATIONS IN BREAST MILK FAT FROM AROUND THE WORLD, 1979-1990

From Tanabe *et al.* (109), pg. 902.

The widespread occurrence of PCBs in breast milk samples from around the world has also caused considerable debate, and concern about the safety of breast feeding. The fear is that where the mother has a high level of PCB contamination the infant may begin life with a burden of toxic pollutants, which will increase with subsequent uptake from dietary or environmental sources.

Mes and Davies (110) reported the levels of PCBs and organochlorine pesticides in human milk samples taken from two women in Canada at various times of the feeding and at different times of the day. The volume and weight of each sample was recorded, and the samples were centrifuged. The fat layer was separated out, and extracted by shaking with 5 % benzene in

acetone. The PCBs were separated from the organochlorine pesticides using a Florisil-silicic acid column chromatography step. The PCBs were eluted with hexane, and the volumes adjusted to 1 ml for analysis. The extracts were analysed by packed column GC-ECD, and Aroclor 1260 was used for quantitation. The results have been expressed on a whole milk basis and a fat basis. On a whole milk basis, the Total PCB concentration found varied between 2.8 ng/g and 16.1 ng/g whole milk. On a fat basis, the Total PCB concentration found varied between 0.207 μg/g and 0.641 μg/g fat. Six milk samples were collected during the morning and midday feedings and four during the evening feeding for each of the two mothers. In the case of one mother, the PCB concentration increased during each feeding, and was highest during the midday feeding. This corresponded with the variation of the fat content of the milk. The results for the second mother were not as clear cut, with fluctuations in the results. These preliminary results showed that sampling at different times during a feeding may lead to different PCB concentrations being found.

The same researchers (111) reported a nationwide survey of PCBs and other compounds in the milk of Canadian mothers. The same extraction and clean-up procedure was used as reported above (110). The extracts were analysed by packed column GC-ECD, and quantified using Aroclor 1260. Confirmation of the identity of the PCBs was done by perchlorination. A total of 100 milk samples from 5 different regions of Canada were analysed. The average concentration of Total PCBs found was 12 ng/g whole milk, with a maximum of 68 ng/g. The results were also reported by region. Ontario and the Western region were found to have the highest average Total PCB levels of 17 ng/g and 15 ng/g, respectively. The Central and Eastern regions had the lowest average Total PCB level, of 8 ng/g.

Rogan *et al.* (107) reported the results of a 1975 survey of various compounds, including PCBs, in mother's milk in the United States. Of the 1,038 samples reported, 9 had no detectable PCBs, 720 had a Total PCB level of less than 0.05 mg/kg in whole milk, and 309 had Total PCB levels of greater than 0.05 mg/kg in whole milk. These 309 mothers had a mean Total PCB concentration of 87 ng/g in whole milk. No extraction or analytical methodology details were

given. Hergenrather *et al.* (112) found approximately the same mean Total PCB level in the breast milk of 12 vegetarian mothers from the U.S.A.

Baluja *et al.* (113) reported the levels of PCBs in human milk samples from Spain. A total of 20 milk samples were collected from mothers in Madrid. The samples were mixed with anhydrous sodium sulphate and extracted in a Soxhlet apparatus for 6 hours. The extracts were cleaned up, and the PCBs separated from the pesticides by Florisil column chromatography. The extracts were analysed by packed column GC-ECD. The 20 samples were found to contain Total PCB concentrations between 0.19 mg/kg and 0.32 mg/kg in whole milk, with an average value of 0.25 mg/kg in whole milk. This value was higher than found in Germany (0.10 mg/kg), U.S.A. (0.04 mg/kg), Sweden (0.02 mg/kg), or Canada (0.01 mg/kg) by other researchers.

Wickström *et al.* (114) reported the levels of PCBs in Finnish human milk samples. 50 milk samples were collected and analysed. 25 g of each milk sample was mixed with sulphuric acid in a separating funnel, and allowed to cool. Hexane was then added, and the funnel was shaken. The aqueous layer was discarded, and the volume of the hexane fraction reduced to about 0.5 ml for analysis. The extracts were analysed by capillary column GC-MS. Total PCB concentrations of between 0.002 mg/kg and 0.041 mg/kg whole milk were found, with an average Total PCB concentration of 0.016 mg/kg whole milk. On a fat basis, the Total PCB concentrations varied between 0.065 mg/kg and 1.2 mg/kg, with an average Total PCB concentration of 0.45 mg/kg milkfat. The PCB concentration in whole milk was compared to the results obtained by a number of other researchers, and found to be similar.

Mes and Lau (115) analysed the distribution of PCB congeners in Canadian human milk samples during lactation. 8 milk samples were taken from one mother at intervals after giving birth, up until 98 days after the birth. The milk samples were centrifuged, and the fat layer extracted with a mixture of acetone and hexane. The extracts were then cleaned up using a Florisil column chromatography step, and analysed by capillary column GC-MS. A standard PCB mixture containing one congener for each level of chlorination was used for quantitation. The standard contained PCBs No. 3, 11, 33, 52, 114, 153, 185, 202, 206, and 209. The results were

presented as the percentage PCB congener distribution on each of 8 days. The PCB congener content remained relatively constant in the breast milk during lactation, except for congeners with 6 and 7 chlorine atoms. The relative amount of hexachlorobiphenyls increased during lactation, while that of heptachlorobiphenyls decreased. After more than 56 days the ratio of hexa- and heptachlorobiphenyls remained relatively constant. Both capillary GC and capillary GC-MS were used to quantify the Total PCB level in each sample. The capillary GC gave Total PCB levels between 9.2 ng/g and 13.9 ng/g whole milk, with an average Total PCB concentration of 11.3 ng/g whole milk. The capillary GC-MS gave levels of between 6.8 ng/g and 11.3 ng/g whole milk, with an average Total PCB concentration of 8.2 ng/g whole milk.

Takei *et al.* (116) analysed PCB levels in human milk samples from Hawaii, U.S.A. A total of 54 milk samples were analysed. The milk samples were extracted with a mixture of acetone and hexane. The PCBs were separated from the lipids by partitioning into acetonitrile. The PCBs were transferred into hexane, and the extracts were cleaned up using Florisil column chromatography, followed by silicic acid column chromatography. The extracts were analysed by packed column GC-ECD, and Aroclor 1254 was used for quantitation. The 54 samples were found to contain Total PCB concentrations of between 0.12 mg/kg and 2.2 mg/kg on a lipid basis. The average Total PCB concentration on a lipid basis was calculated as 0.80 mg/kg, and this was compared with a U.S. EPA study result of 0.97 mg/kg, for 102 human milk samples from mainland U.S.A. These two results were not significantly different, showing that female residents of Hawaii had levels of PCBs in their breast milk similar to women from mainland U.S.A.

Schulte and Malisch (117) reported the levels of 55 individual PCB congeners in German breast milk samples. Eight breast milk samples were analysed. 10 g of each sample was extracted and cleaned up. The solvent was evaporated, the extract dissolved in hexane, and the PCBs fractionated by silica gel column chromatography. The extracts were analysed by capillary column GC-ECD and GC-MS. The results for all of the 55 individual PCB congeners were reported, and a mean Total PCB concentration for the eight milk samples of 1.1 mg/kg fat was calculated from the individual congener results. The Total PCB concentrations for each of the eight milk samples

calculated in this work were compared with a method of quantitation using just PCBs No. 138, 153, and 180. This quantitation method gave Total PCB concentrations that were twice those found by using the 55 individual PCB congeners, and therefore a conversion factor was needed to correct the results obtained by using just PCBs No. 138, 153, and 180 to calculate Total PCB concentrations.

Mes *et al.* (118) carried on the work of Mes and Lau (115) by analysing changes in the levels of PCBs in breast milk samples and blood taken from Canadian women during lactation. Breast milk samples from 16 women were analysed for PCBs at eight intervals during a 98 day lactation period. For each sample, 25 g of human milk was extracted with a mixture of acetone and benzene. The PCBs in the extracts were fractionated from the other extractants using a combined Florisil-silicic acid column chromatography step. The extracts were analysed by capillary column GC-ECD, and a standard solution of Aroclor 1260 was used for quantitation. The Total PCB levels at 7, 14, 28, 42, 56, 70, 84, and 96 days after the birth were reported. The results varied from 22.8 ng/g whole milk to 29.7 ng/g whole milk. There was no evidence of the Total PCB concentration in the breast milk increasing or decreasing during the 96 days of investigation. The total amounts of PCBs transferred to the infant at intervals during the 96 days were calculated, and displayed graphically. It was calculated that 1.6 μg/g infant body fat could theoretically have been passed to the infant over the 96 days.

The same research group of Mes *et al.* (119) analysed the levels of PCBs in a total of 210 breast milk samples from 5 different regions across Canada. 10 g aliquots of each milk sample were centrifuged, and the fat layer was extracted by shaking with a mixture of acetone and benzene for 2 minutes. The extract was filtered, the solvent evaporated off, and the extract reconstituted in hexane. This was filtered through anhydrous sodium sulphate. An aliquot of the hexane extract, containing not more than 250 mg of lipid, was evaporated to less than 1 ml under nitrogen, and cleaned up on a combined Florisil-silicic acid column. The extracts were analysed and quantified by capillary column GC-ECD, and peak identity confirmed by capillary column GC-MS. Aroclor 1260 was used as the quantitation standard. The average Total PCB concentration found in the

210 samples was 26 ng/g milk or 697 ng/g milkfat, with a maximum observed value of 80 ng/g milk or 3,022 ng/g milkfat. The results were compared with earlier Canadian results and work from other countries. The average result of 26 ng/g milk was higher than those found in previous Canadian studies, but this apparent increase was due to improved analytical and sample collection techniques, rather than a real increase in the PCB levels in Canadian breast milk, which had not been found by other researchers. The average result of 697 ng/g milkfat was in agreement with levels found by researchers in other industrialised countries. The results for the 5 different regions of Canada were found to be very similar.

Mes *et al.* (120) analysed a number of individual PCB congeners in human milk samples from Canada. The breast milk samples were extracted and cleaned up using the same method as reported previously by the same authors (119). The extracts were analysed and quantified by capillary column GC-ECD, and peak identity confirmed by capillary column GC-MS. A total of 14 individual PCB congeners were analysed, PCBs No. 28, 52, 66, 74, 101, 105, 118, 138, 151, 153, 156, 180, 183, and 187. A total of 75 breast milk samples were analysed. All 14 individual PCB congeners were found in the milk samples, with PCBs No. 74, 118, 138, 153, and 180 being the most abundant, contributing 75 % of all the PCB congeners measured. The mean Total PCB concentration, from the 14 individual congeners, for the 75 breast milk samples was 15.9 ng/g. The results for PCBs No. 105, 138, 153, 156, and 180 were compared with those from an American study, and found to be in broad agreement.

The same research group also analysed pooled Canadian milk samples for 29 individual PCB congeners (66), including most of the 14 congeners listed above (120). PCBs No. 74, 118, 138, 153, and 180 were again found to be the most abundant congeners. Davies and Mes (121) compared the levels of PCBs in breast milk from the general and indigenous Canadian populations. A total of 18 milk samples were collected from the indigenous population of Canada. The same extraction and clean-up methods were used as previously described (119), and the extracts were analysed by capillary column GC-ECD. The same 14 individual PCB congeners were analysed as used above (120). The combined Total PCB congener level on a whole milk basis was 15.9 ng/g

nationally and 12.4 ng/g for the indigenous population. As previously (120), the most abundant congeners were found to be PCBs No. 74, 118, 138, 153, and 180.

Dommarco *et al.* (122) determined the levels of PCBs in 65 human milk samples from Rome, Italy. The samples were frozen after collection. The milk samples were extracted by centrifuging with a mixture of hexane, acetonitrile, and ethanol. The extracts were evaporated to dryness, and then dissolved in hexane. Next, the extracts were cleaned up by Florisil column chromatography, and analysed by packed column GC-ECD. A 1:1 mixture of Fenchlor 1254 and 1260 was used for quantitation. PCBs were found in all 65 milk samples, with a mean Total PCB concentration of 70 ng/g whole milk. This value was found to be similar to that reported in an earlier Italian study, and in other studies from different countries.

Mussalo-Rauhamaa *et al.* (123) analysed the levels of PCBs in 183 breast milk samples from 165 women, living in different parts of Finland. A 15-20 g sample of milk was extracted with hexane, and cleaned up with concentrated sulphuric acid. The extracts were analysed by capillary column GC-MS, and a solution of Clophen A60 was used for quantitation. A mean Total PCB concentration of 0.93 mg/kg fat was reported. The individual results for the different women were statistically compared with a number of different factors, e.g. age, diet, but no positive correlations were found.

Skaare *et al.* (124) analysed the levels of PCBs and organochlorine compounds in breast milk samples from 43 Norwegian women. The milk samples were extracted with a mixture of acetone and hexane by ultrasonic disintegration. The extract was split into two aliquots, which were treated with concentrated sulphuric acid and methanolic potassium hydroxide, respectively. The extracts were analysed by packed column GC-ECD, using two different columns. The PCBs were quantified using Aroclor 1254 as a standard. The results were reported in three groups. 16 of the women gave birth by a Caesarian operation (Group 1), 20 of the women gave birth without the need for such an operation (Group 2), and 7 of the women were recent immigrants to Norway from India or Pakistan, and gave birth without an operation (Group 3). The average Total PCB levels for Groups 1 and 2 were 20 ng/g whole milk and 23 ng/g whole milk, respectively. No

determination of PCB concentrations was done on the milk samples from the women in Group 3. In addition, no significant difference in levels of PCBs in Norwegian breast milk was seen between 1976 and 1982.

Schecter *et al.* (125) analysed the levels of PCBs, and a number of other organic compounds, in human milk samples from the United States, Thailand, Vietnam, and Germany. The levels of 9 individual PCB congeners (PCBs No. 28, 52, 101, 118, 138, 153, 170, 180, and 194) were reported in 190 human milk samples from the four countries. PCBs No. 138, 153, and 180 were detected in all of the samples, while the other 7 congeners were only found in some of the samples. The average total level of the 9 individual PCB congeners was highest in the German milk samples at 750 ng/g in milkfat, followed by the American milk samples at 145 ng/g, respectively. The milk samples from a rural area of Vietnam showed the lowest average Total PCB concentration of the 9 individual congeners of 28 ppb, while those from other areas of Vietnam showed a concentration of 67 ng/g, and those from Thailand showed a concentration of 55 ng/g. These results clearly showed that PCBs, as well as PCDDs, PCDFs, and pesticides, were present in the breast milk of mothers from various countries around the world.

Dewailly *et al.* (126) compared the levels of PCBs in milk samples from 24 Inuit women and 48 Caucasian women from Canada. The milk samples were subjected to alkaline hydrolysis to eliminate fats. The PCBs were, then, extracted with a mixture of hexane and ether. The extracts were cleaned up by Florisil column chromatography, and analysed by capillary column GC-ECD. Aroclor 1260 was used as the quantitation standard. The mean Total PCB concentrations were found to be 111.3 μg/l whole milk for the Inuit women, and 28.4 μg/l whole milk for the Caucasian women. The PCB level among the Inuit women was almost 5 times that of the Caucasian women, and among the highest PCB levels ever reported. A high consumption of fish and sea mammals was reported to be the probable main route of intake of PCBs for the Inuit women.

Tanabe *et al.* (109) analysed the levels of PCBs in human breast milk samples from Southern India. A total of 25 milk samples were collected from rural, urban, and cosmopolitan areas in Tamil Nadu state, in Southern India. The milk samples were extracted with mixed solvents, and

transferred to hexane. The volume of solvent was reduced using a Kuderna-Danish apparatus, and the extracts were cleaned up by Florisil column chromatography, followed by silica gel column chromatography. The extracts were analysed by capillary column GC-ECD. The mean Total PCB concentration found in the breast milk samples from all the areas was 120 ng/g on a fat weight basis, with the lowest levels in samples from rural areas. The PCB levels in Indian breast milk were lower than those previously reported in other countries.

Krauthacker (127) analysed the levels of PCBs, and pesticides, in human milk from mothers in the Northern Adriatic area of former Yugoslavia. A total of 53 milk samples were collected from 43 mothers in two different locations. The milk samples were extracted with a mixture of chloroform and methanol, and cleaned up with concentrated sulphuric acid. The extracts were analysed by packed column GC-ECD, and Aroclor 1260 was used for quantitation. The mean Total PCB concentrations for the two different locations were reported as 15 ng/g and 9 ng/g on a whole milk basis.

Norén and Lundén (128) analysed the levels of PCBs, PCDDs, and PCDFs in human milk samples from Sweden. The milk samples were collected between 1972 and 1989, and pooled samples, consisting of milk from 10-20 mothers, were analysed. 10ml of each milk sample was extracted with Lipidex 5000, and cleaned up with alumina, silica gel, and activated charcoal columns. The extracts were analysed by capillary column GC-ECD and GC-MS. The average Total PCB levels from 1976 to 1989 varied between 0.93 μg/g fat and 0.60 μg/g fat. The levels of PCBs No. 28, 105, 118, 138, 153, 156, and 180 were reported relative to the Total PCB concentrations. In addition, the levels of the three coplanar PCB congeners (PCBs No. 77, 126, 169) were reported. The concentration of PCB No. 77 varied from 27 pg/g to 76 pg/g, PCB No. 126 varied from 98 pg/g to 298 pg/g, and PCB No. 169 varied from 43 pg/g to 74 pg/g. All of these results were reported on a fat basis. The levels of Total PCBs and coplanar congeners fell between 1972 and 1985, but were fairly constant thereafter.

Bordet *et al.* (129) analysed the levels of PCBs and organochlorine pesticides in human milk samples from France. 20 human milk samples were analysed for the presence of 17 individual

PCB congeners, PCBs No. 28, 44, 52, 66, 77, 99, 101, 105, 118, 126, 138, 153, 156, 169, 180, 187, and 203. No details of the extraction methodology was given. The extracted samples were analysed by capillary column GC-ECD. The concentrations of all 17 PCB congeners in ng/g of milkfat in each of the 20 milk samples were reported. The most frequently found congeners were PCBs No. 118, 138, 153, and 180, at average concentrations of between 37 ng/g and 103 ng/g of milkfat. The coplanar PCB congeners, PCBs No. 77, 126, 169, were also present, at concentrations between 29 ng/g and 45 ng/g of milkfat.

Hernández *et al.* (130) analysed the levels of PCBs and organochlorine pesticides in human milk samples from Madrid, Spain. 51 milk samples were analysed in this study. The milk samples were dispersed in anhydrous sodium sulphate, and extracted with a mixture of acetone and hexane. The extracts were evaporated under nitrogen, and the compounds of interest isolated by liquid-liquid partitioning. Next the extracts were cleaned up by Florisil column chromatography. The extracts were analysed by capillary column GC-ECD. The mean Total PCB concentration in the 51 milk samples was 61 ng/g on a whole milk basis. This result was lower than that reported by Baluja *et al.* (113) in 1982. The Total PCB level was subsequently compared with results from other nations, and was found to be in line with those of most industrialised countries.

Duarte-Davidson *et al.* (131) investigated the levels of PCBs and organochlorine pesticides in human milk samples from Wales. 115 milk samples were analysed. The samples were freeze dried, and 2 g of each sample was then mixed with anhydrous sodium sulphate to remove any water that was present. The samples were extracted with hexane, and cleaned up with sulphuric acid. Alumina column chromatography was then used to further clean up the samples. The samples were analysed by GC-ECD, with some identity confirmation by GC-MS. The samples were screened for 50 individual PCB congeners, of which 24 were found in most samples. PCBs No. 28, 138, 153, and 180 accounted for approximately 50 % of the Total PCBs found. Total PCB concentrations of between 2 ng/g whole milk and 70 ng/g whole milk were found, with a mean value of 12 ng/g whole milk. This represented a PCB intake by the infant of 1.4 μg/kg/day. A correlation was found for an increase in Total PCB concentration with an increase in the age of

the individual mothers. A correlation was also found for a decrease in Total PCB concentration with the total period of breast feeding.

Dewailly *et al.* (132) investigated the levels of coplanar PCB congeners, PCBs No. 77, 126, and 169, PCDFs, and PCDDs in human milk samples from Québec, Canada. The determination of these compounds was used to evaluate the toxic potential of breast milk for infants. Milk samples were collected from 96 women, the milk samples were pooled, and 16 pools of six samples each were prepared. The milk samples were mixed with an aqueous solution of sodium oxalate, ethyl ether, and ethanol, and then extracted with hexane. The extracts were cleaned up using a sulphuric acid-silica gel slurry, followed by a neutral/acid-silica gel column chromatography step. The coplanar PCB congeners were separated from interferences using alumina and Carbopack C/Celite columns. The coplanar PCBs were analysed by capillary column GC-MS, and the other PCBs analysed by capillary column GC-ECD. Aroclor 1260 was used to quantify the Total PCB levels found. The three coplanar PCB congeners were detected in all 16 milk samples, with mean concentrations of 8.1 pg/g, 80.5 pg/g, and 32.7 pg/g milkfat for PCBs No. 77, 126, and 169, respectively. These values were slightly lower than those found in Sweden (129). The levels of three mono-ortho PCB congeners, PCBs No. 105, 114, and 156, were also reported. The mean concentrations of these three congeners were 4,400 pg/g, 17,400 pg/g, and 6,200 pg/g milkfat for PCBs No. 105, 114, and 156, respectively. Using a set of toxic equivalence factors (TEFs), Dewailly *et al.* found that the PCBs represented a higher risk than PCDFs and PCDDs. Additionally, mono-ortho PCBs represented an important part of the toxicity of PCB mixtures.

Hong *et al.* (133) analysed the levels of mono-ortho and non-ortho substituted PCBs in human milk from the U.S.A. 15 g of each milk sample was extracted by centrifuging with ethanol and hexane. The volume of the extract was reduced using a Kuderna-Danish apparatus. The extract was cleaned up by Florisil column chromatography. The planar PCBs were isolated from the non-planar PCBs using a porous graphitic carbon (PGC) HPLC column, with hexane as the eluting solvent. The extracts were analysed by capillary column GC-ECD. A standard solution containing 4 non-ortho substituted PCB congeners (PCBs No. 77, 81, 126, and 169) and 8 mono-

ortho substituted PCB congeners (PCBs No. 105, 114, 118, 123, 156, 157, 167, and 189) was used for quantitation. The levels of each of these 12 individual PCB congeners in 5 breast milk samples from upstate New York were reported, with the total level of these PCB congeners ranging from 527 pg/g to 1,770 pg/g of whole milk.

Steinwandter (70) analysed the levels of non-ortho and mono-ortho substituted PCB congeners in human milk samples from Germany. Human milk samples were obtained from six mothers. The milk samples were extracted with a mixture of acetone and petroleum ether, using a column chromatography method. The extracts were cleaned up by a silica gel column chromatography step. The coplanar PCB congeners were isolated from the other PCB congeners using an activated carbon HPLC micro column, with petroleum ether and toluene used as the eluting solvents. The extracts were analysed by capillary column GC-MS. 5 non-ortho substituted PCB congeners (PCBs No. 77, 79, 81, 126, and 169) and 15 mono-ortho substituted PCB congeners (PCBs No. 28, 33, 52, 56+60, 65, 70, 72, 105, 108, 114, 118, 156, 157, 167, and 189) were found in the human milk samples. PCB No. 126 at 360 pg/g whole milk was the non-ortho substituted PCB congener found in the highest concentration, while PCB No. 118 at 20,600 pg/g whole milk was the mono-ortho substituted PCB congener found in the highest concentration. With the use of toxic equivalence factors, Steinwandter also found that the PCBs represented a higher risk than PCDDs and PCDFs in human milk. This was in agreement with Dewailly *et al.* (132).

6.2: RESULTS:

A total of 23 breast milk samples were analysed, as outlined in Chapter 2. All of the samples were analysed by capillary column GC-MS. The concentrations of the PCBs found in the breast milk samples have been reported by homologue. All of the results shown were averaged from duplicate analyses of each breast milk extract. The results for all 23 breast milk samples are shown in Table 6.4.

PATIENT No.	PCB CONCENTRATION IN mg/kg WHOLE MILK						
	Cl_2	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Total PCBs
1	0	0.0011	0.0028	0.0012	0.0022	0	0.0072
	0	15.3	38.9	16.7	30.6	0	
3	0.0014	0.0031	0.0027	0.0041	0.0029	0.0008	0.0149
	9.4	20.8	18.1	27.5	19.5	5.4	
4	0	0.0011	0.0009	0.0033	0.0051	0	0.0104
	0	10.6	8.7	31.7	49.0	0	
5	0.0058	0.0229	0.0196	0.0126	0.0053	0	0.0663
	8.7	34.5	29.6	19.0	8.0	0	
8	0	0.0028	0.0036	0	0.0035	0.0009	0.0109
	0	25.7	33.0	0	32.1	8.3	
9	0	0.0031	0.0033	0.0030	0.0079	0.0019	0.0191
	0	16.2	17.3	15.7	41.4	9.9	
10	0	0.0011	0.0032	0.0013	0.0046	0	0.0102
	0	10.8	31.4	12.7	45.1	0	
12	0	0.0019	0.0036	0.0030	0.0091	0.0012	0.0188
	0	10.1	19.1	16.0	48.4	6.4	
13	0.0032	0.0075	0.0063	0.0061	0.0063	0.0010	0.0304
	10.5	24.7	20.7	20.1	20.7	3.3	
14a	0	0.0030	0.0035	0.0014	0.0008	0	0.0088
	0	34.1	39.8	15.9	9.1	0	
15	0.0053	0.0141	0.0091	0.0083	0.0045	0	0.0413
	12.8	34.1	22.0	20.0	10.9	0	
17	0.0032	0.0094	0.0046	0.0052	0.0026	0.0009	0.0259
	12.3	36.2	17.7	20.0	10.0	3.5	
18	0	0.0019	0.0019	0.0031	0.0019	0	0.0088
	0	21.6	21.6	35.2	21.6	0	
19	0	0.0043	0.0043	0.0028	0.0023	0.0001	0.0139
	0	30.9	30.9	20.1	16.5	0.7	
21b	0	0.0014	0.0054	0.0024	0.0009	0	0.0101
	0	13.9	53.5	23.8	8.9	0	
22	0	0.0012	0.0035	0.0017	0	0	0.0065
	0	18.5	53.8	26.2	0	0	
24	0.0036	0.0063	0.0071	0.0058	0.0040	0	0.0268
	13.4	23.5	26.5	21.6	14.9	0	
25	0.0013	0.0053	0.0049	0.0036	0.0065	0.0006	0.0223
	5.8	23.8	22.0	16.1	29.1	2.7	

26	0.0012	0.0054	0.0061	0.0039	0.0024	0	0.0189
	6.3	28.6	32.3	20.6	12.7	0	
27	0.0010	0.0047	0.0037	0.0031	0.0080	0.0024	0.0230
	4.3	20.4	16.1	13.5	34.8	10.9	
28	0.0009	0.0051	0.0042	0.0034	0.0039	0.0004	0.0179
	5.0	28.5	23.5	19.0	21.8	2.2	
29	0.0015	0.0091	0.0060	0.0070	0.0093	0.0006	0.0335
	4.5	27.2	17.9	20.9	27.8	1.8	
30	0	0.0036	0.0054	0.0033	0.0011	0	0.0134
	0	26.9	40.3	24.6	8.2	0	
MEAN	0.0012	0.0052	0.0050	0.0039	0.0041	0.0005	0.0199
(n = 23)	6.0	26.1	25.1	19.6	20.6	2.5	

N.B. The lower numbers in each box represent the PCB concentration expressed as a % w/w of the Total PCB concentration.

TABLE 6.4: PCB CONCENTRATIONS BY HOMOLOGUE IN 23 BREAST MILK SAMPLES

Dichlorobiphenyls through to heptachlorobiphenyls were detected in the 23 samples. All 23 breast milk samples were found to contain quantifiable amounts of PCBs. The numbers below the concentration value for each homologue are the percentages of that homologue in the Total PCB concentration result.

The Total PCB concentrations found in the 23 breast milk samples ranged from 0.007 mg/kg whole milk to 0.066 mg/kg whole milk, with a mean value of 0.020 mg/kg whole milk. The mean concentrations for each level of chlorination are shown in Diagram 6.1.

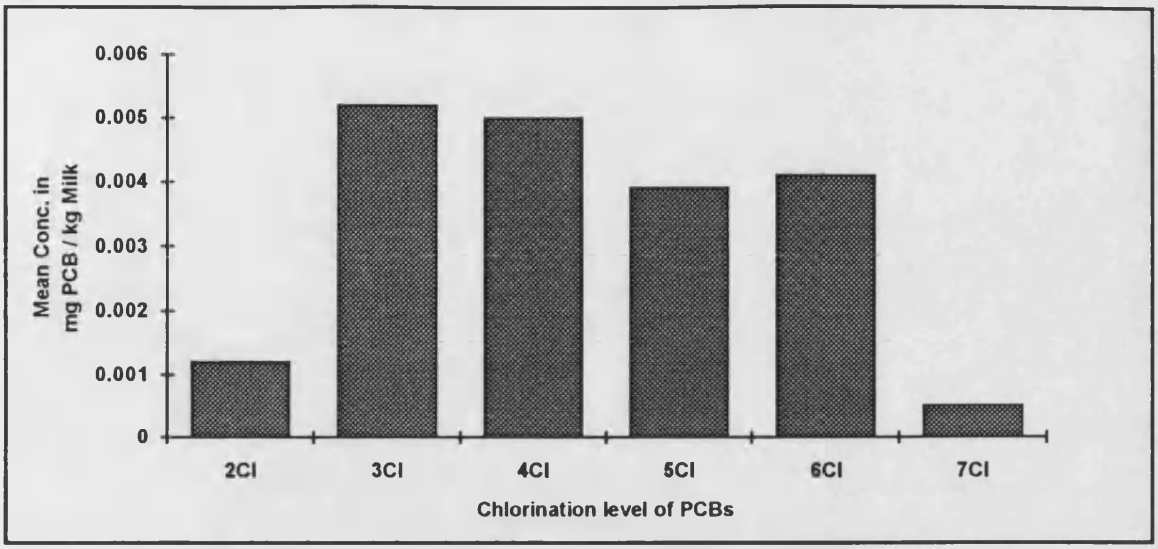


DIAGRAM 6.1: MEAN PCB CONCENTRATIONS BY HOMOLOGUE IN 23 BREAST MILK SAMPLES

Diagram 6.1 shows that the average concentrations for the trichloro through to hexachlorobiphenyls were very similar, ranging from 0.0039 mg/kg Whole Milk to 0.0052 mg/kg Whole Milk. The results for the individual mothers showed that not all of them contained this pattern of PCB congeners in their breast milk. This is discussed below in Section 6.3.

The frequency of breast milk samples by level of Total PCB concentration is shown in Diagram 6.2.

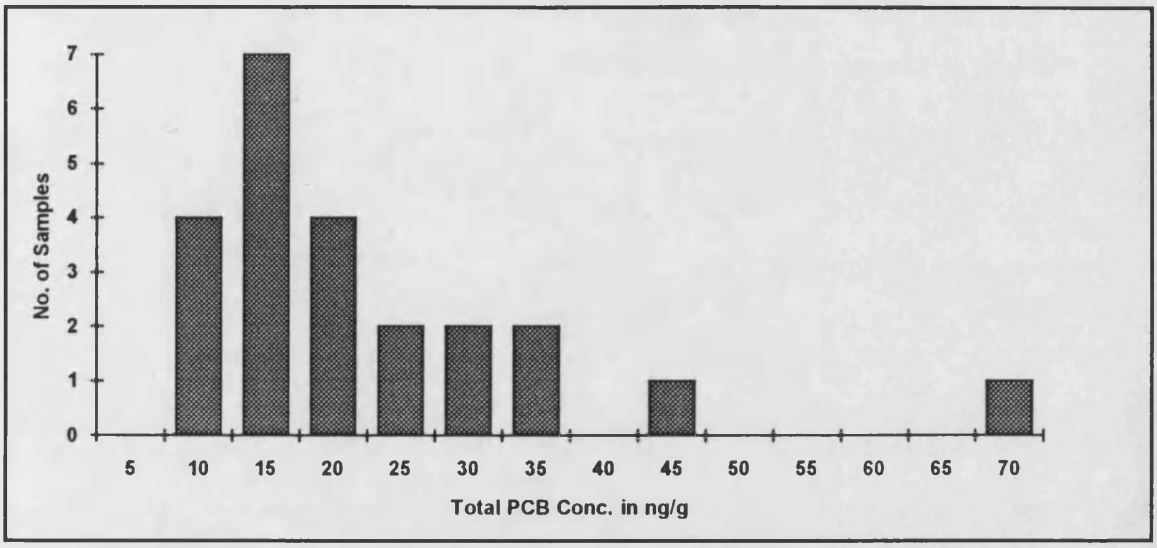


DIAGRAM 6.2: FREQUENCY OF BREAST MILK SAMPLES BY TOTAL PCB CONCENTRATION

Diagram 6.2 shows that 21 of the 23 mothers contained Total PCB concentrations of between 10 ng/g Whole Milk and 35 ng/g Whole Milk. Only 2 of the mothers showed higher Total PCB concentrations. In addition, 15 of the 23 mothers showed Total PCB results of between 10 ng/g Whole Milk and 20 ng/g Whole Milk.

6.3: DISCUSSION:

A number of different patterns of PCB residues, expressed by homologue, have been found in the 23 breast milk samples analysed, Diagram 6.3.

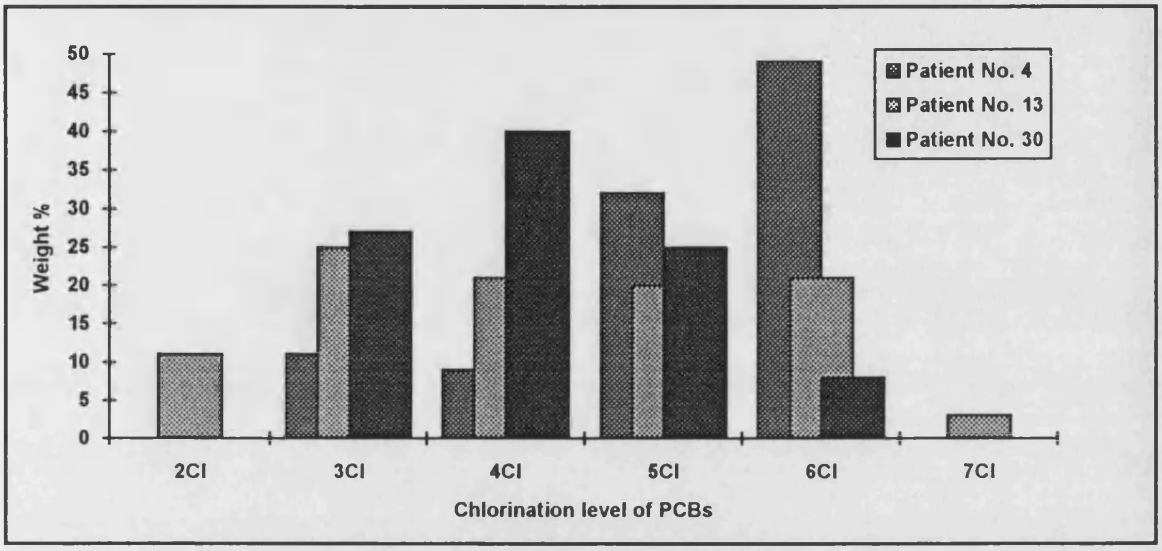


DIAGRAM 6.3: EXAMPLES OF THREE DIFFERENT PCB PROFILES FOUND IN THE BREAST MILK SAMPLES

The women will have been subject to PCB contamination throughout their lives, from their daily diets and from the environment, which will have accumulated in the breast milk. Over a long period of time, the lower chlorinated PCBs will be metabolised faster than higher chlorinated congeners. Therefore, the profile of Patient No. 4, which shows a higher percentage of the higher chlorinated PCBs indicates a greater degree of metabolism of the lower chlorinated PCBs than the profile of Patient No. 30. Similarly, the profile of Patient No. 13 indicates a higher degree of metabolism of the lower chlorinated PCBs than the profile of Patient No. 30. The higher chlorinated PCBs tend to accumulate in the fatty regions of the body, and are expressed in the breast milk. The profiles of the PCB patterns for each mother can, therefore, give some information about the level of metabolism of PCBs that has occurred. Of course, individual

women may have come into contact with PCBs, or other organic compounds, at work, or a local spillage or leakage of PCBs may have occurred. Differences may also have been caused by the type of diet the individual women were eating. Unfortunately, no detailed information about the individual mothers could be obtained. Therefore, it was not possible to draw any definite conclusions about the different PCB patterns observed in the breast milk samples. All 23 of the breast milk samples contained PCBs with one of the three different patterns shown in Diagram 6.3.

The mean Total PCB concentration of 20 ng/g whole milk was similar to that found in an earlier study of British human breast milk samples, reported by MAFF (86). In this earlier study, 102 breast milk samples were analysed between 1979 and 1980, and a mean Total PCB concentration of 18 ng/g whole milk was reported. Similarly, Duarte-Davidson *et al.* (131) reported a mean Total PCB concentration of 12 ng/g for 115 Welsh breast milk samples.

The mean Total PCB concentration of 0.020 mg/kg whole milk was compared with the values obtained by other researchers. The mean Total PCB concentrations found in breast milk in 14 of the references reviewed above are listed in Table 6.5, along with the mean result for the present study.

REF No.	No. of Subjects	Mean Total PCB Concentration (ng/g)
107. Rogan *et al.*	309	87
111. Mes & Davies	100	12
113. Baluja *et al.*	20	250
114. Wickström *et al.*	50	16
115. Mes & Lau	8	11.3
118. Mes *et al.*	128	22.8-29.7
119. Mes *et al.*	210	26
120. Mes *et al.*	75	15.9
121. Davies & Mes	18	12.4
122. Dommarco *et al.*	65	70
124. Skaare *et al.*	16, 20	20, 23
127. Krauthacker	33, 20	15, 9
130. Hernández *et al.*	51	61
131. Duarte-Davidson *et al.*	115	12
Current Work	23	20.0

TABLE 6.5: COMPARISON OF TOTAL PCB CONCENTRATIONS IN WHOLE BREAST MILK SAMPLES

These values were reported between 1975 and 1994, and ranged from 9 ng/g whole milk to 250 ng/g whole milk. 10 of the 14 references reported mean Total PCB concentrations in whole breast milk of between 9 ng/g whole milk and 30 ng/g whole milk.

The results obtained in this study indicate that PCBs are probably present in most breast milk samples at quantifiable concentrations. For an infant ingesting, on average, approximately 150 ml of milk per kilogram of body weight each day, the mean Total PCB concentration of 0.020 mg/kg whole milk represents a PCB intake by the infant of 3.0 μg/kg/day. The recommended maximum intake of PCBs in a 1985 World Health Organisation (WHO) consultation paper, on organohalogens in human milk, was 1.0 μg/kg/day (134). It must be remembered, however, that the significance of these Total PCB values is difficult to assess because of the wide variations in the toxicities of different PCB congeners.

CHAPTER 7: THE USE OF SUPERCRITICAL FLUIDS FOR THE EXTRACTION AND ANALYSIS OF PCBS

7.1: INTRODUCTION TO SUPERCRITICAL FLUIDS:

The sample preparation methodology commonly used in analytical laboratories worldwide usually involves either Soxhlet extraction or sonication, followed by column chromatography as the clean-up step. These methods are time consuming, and use large volumes of toxic, flammable organic solvents. Typically two-thirds of the analysis time can be spent on sample preparation, and sample preparation can also account for at least one-third of the error generated during the performance of an analytical method (135). Concern about the use of large amounts of solvent, coupled with the cost and environmental dangers of waste solvent disposal, have resulted in an urgent need to find safer extraction methods. This has led to a huge volume of research into the properties and possible uses of supercritical fluids, both for extraction (supercritical fluid extraction, SFE) and chromatography (supercritical fluid chromatography, SFC).

The high solvating power of supercritical fluids was reported in the last century by Hannay and Hogarth (136), less than a decade after the discovery of the critical phenomenon, and during a period of intense activity surrounding the critical state of gases. Subsequently, this solvating power was observed by an array of researchers in the fields of engineering and geology, as well as by chemists. The feasibility of using supercritical fluids for analytical chromatography was first demonstrated by Klesper *et al.* in 1962 (137). The use of supercritical fluids for the extraction of target compounds was first reported by Zosel in 1969 (138). Since the 1960's, a large number of researchers have used various fluids as supercritical fluids, with both GC and LC instrumentation. A wide range of compounds have been investigated using supercritical fluids. As the full potential of supercritical fluids in analytical chemistry has begun to emerge, so there has been a huge increase in the research efforts into supercritical fluids.

7.1.1: THE PHYSICAL PROPERTIES OF A SUPERCRITICAL FLUID:

A fluid is in its supercritical state when both its temperature and pressure are above their critical values. If only one of these two parameters fulfills this condition, it is said to be in its subcritical state. Therefore, in the region above the critical temperature the substance cannot be liquefied by increasing the pressure, and there is no possible phase transfer from gas to liquid, or vice versa, see Diagram 7.1.

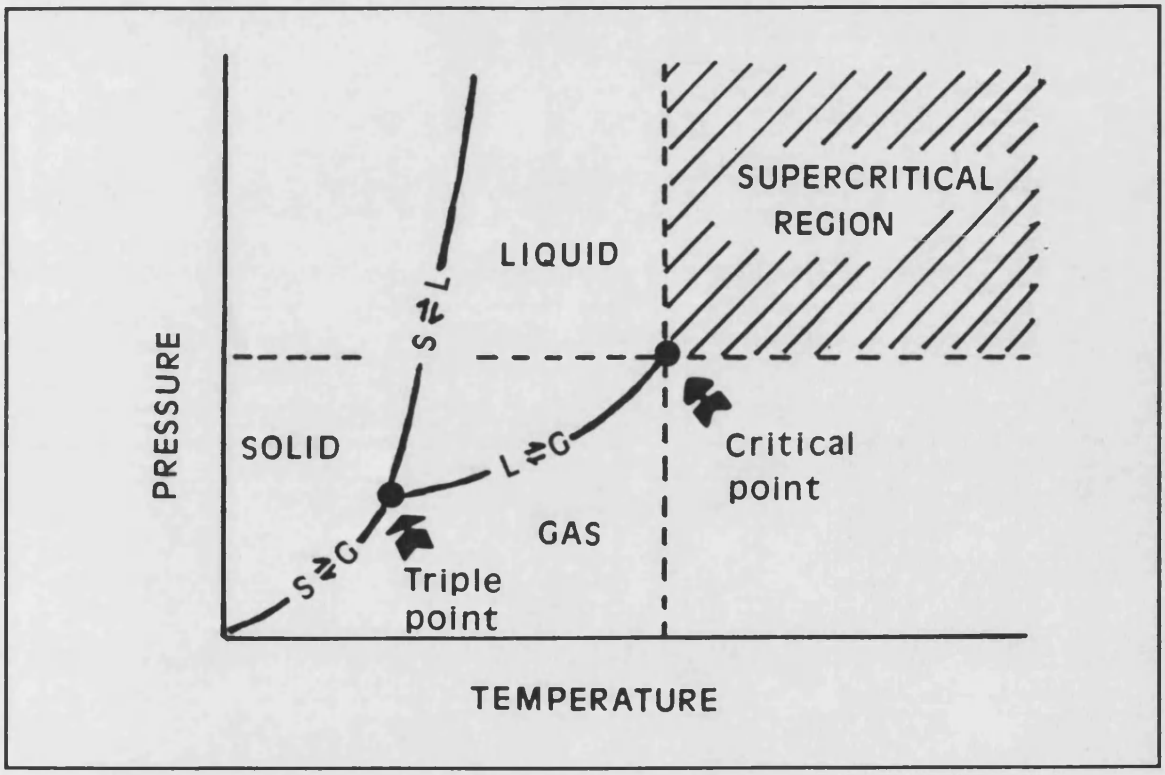


DIAGRAM 7.1: PHASE DIAGRAM SHOWING SUPERCRITICAL REGION

Supercritical fluids possess unique physicochemical properties which make them attractive for a variety of extraction and separation processes. The viscosities of supercritical fluids are similar to those of gases, 5 to 20 times lower than those of liquids, providing the potential for faster extractions than with classical liquid phases. Additionally, the zero surface tension of supercritical fluids allows for efficient penetration into macroporous materials. Supercritical fluids have

densities 100 to 1000 times greater than those of gases and, therefore, have solvent strengths close to those of liquids.

The solvent strength of a supercritical fluid is directly related to its density and, thus, the solvating ability of a particular supercritical fluid towards a particular species can easily be modified by changing the extraction pressure, and to a lesser extent, the temperature. This allows the extraction conditions to be optimised for a target analyte, and class-selective extractions of particular compound classes can be obtained by extracting the same sample at different pressures (139).

Supercritical fluids with widely varying polarities are available, and the polarity of a supercritical fluid can also be controlled by the addition of solvent modifiers (140). Very pure supercritical fluids are available that are relatively inert, inexpensive, and have low critical temperatures (e.g. CO_2, nitrous oxide, and ethane have critical temperatures of 31 ^{o}C, 36.5 ^{o}C, and 32 ^{o}C, respectively). The latter allows extractions to be performed under conditions that protect thermally unstable analytes. Several supercritical fluids are gases at ambient conditions, which greatly simplifies the analyte collection and concentration steps (139). Supercritical CO_2 has been by far the most widely used supercritical fluid. This is because CO_2 is a chemically inert, inexpensive compound that is neither toxic nor flammable, and has an easily accessible critical point at 74.4 kgf/cm^2 and 31.1 ^{o}C. Carbon dioxide is available in pure form and, if desired, can be further purified. Supercritical CO_2 vapourises on release to the atmosphere, enabling extracted solutes to be easily isolated for further analysis, and facilitates the coupling of supercritical fluid extraction (SFE) with a number of chromatographic techniques. Many other supercritical fluids have been used as well as CO_2, e.g. N_2O, SF_6, CH_3OH, H_2O, $CHClF_2$, and some alkanes (141). The critical properties of various fluids are given in Table 7.1.

FLUID	CRITICAL PROPERTIES	
	PRESSURE P_c (kgf/cm^2)	TEMP T_c (^oC)
CO_2	74.4	31.1
Xe	59.9	16.8
Methanol	82.5	240.0
Methane	46.9	- 82.0
Propane	43.2	97.0
Pentane	34.4	196.6
$CClF_3$	39.5	28.9
$CHClF_2$	50.6	96.0
SF_6	38.3	45.6
SO_2	80.6	158.0
N_2O	74.9	36.4
NH_3	118.6	132.4
Benzene	49.9	289.0
H_2O	224.3	374.0

TABLE 7.1: CRITICAL PROPERTIES OF SOME FLUIDS

A table showing conversion factors for the different units of pressure is also provided, Table 7.2.

ORIGINAL UNIT	CONVERTS TO
1 Pa	1 N/m^2 = 10 dyn/cm^2
1 torr	133.322 Pa
1 atm	760 torr = 101.325 kPa
1 bar	100 kPa = 0.9869 atm
1 kg/cm^2	98.062 kPa = 0.9678 atm
1 p.s.i.	0.070309 kg/cm^2

TABLE 7.2: CONVERSION TABLE FOR PRESSURE UNITS

7.1.2: INTRODUCTION TO SUPERCRITICAL FLUID EXTRACTION:

Supercritical fluid extraction (SFE) was first demonstrated as an industrial process by Zosel, at the Max Planck Institute for Coal Research in 1969 (138). SFE developed into an important industrial-scale extraction technique as an alternative to distillation, and traditional solvent extractions. There have been a wide range of reports, by a large number of research groups, on the development of SFE as an industrial scale process, and SFE has found numerous uses in engineering (142). In the 1970's, foodstuffs became the centre of interest for SFE, and there are reports on the use of SFE for the decaffeination of coffee, and in the extraction of hops, tobacco,

and spices. In West Germany in 1979, Hag built the first large-scale production plant using SFE, to remove caffeine from green coffee beans.

In the 1980s, increasing attention was given to analytical-scale SFE as a means of sample preparation in analytical chemistry. SFE can potentially provide more rapid, efficient and selective extractions than conventional extraction techniques. A variety of organic compounds have been extracted from different matrices using supercritical fluids. Extraction using supercritical fluids can achieve better efficiencies than conventional Soxhlet extraction, in a much shorter time period, for some analytes and matrices. This has been demonstrated for the extraction of polycyclic aromatic hydrocarbons from environmental solids by Hawthorne and Miller (143). SFE also minimises the use of toxic organic solvents, and the need for concentration steps before analysis of the extracts. SFE can be optimised for a particular extraction because the solvent strength of a supercritical fluid is directly related to its density, which can be easily modified by changing the extraction pressure, and to a lesser extent, the temperature.

Snyder *et al.* (144) compared SFE with classical sonication and Soxhlet extraction techniques for organochlorine and organophosphate pesticides from soils. SFE with CO_2, modified with 3 % methanol, at 361.6 kgf/cm^2 and 50 ^oC, was found to give similar recoveries of the pesticides, when compared with the sonication and Soxhlet methods. Soxhlet extraction took 22 hours, and sonication took 2 hours to complete, whereas SFE could be completed in less than one hour. SFE also required less solvent than the two classical methods, and no concentration of the solvent extracts prior to analysis was needed. Some instrumental drawbacks of SFE were noted.

7.1.2.1: The Supercritical Fluid Extraction Of PCBs:

Hawthorne and Miller (145) demonstrated the extraction of PCBs, as an Aroclor 1254 spike, from river sediment using SFE, coupled with GC-ECD. Nitrous oxide, N_2O, at 310 kgf/cm^2 and 45 ^oC was used to extract a spike of 8.0 μg/g Aroclor 1254, on a 10 mg sample of river sediment.

The resultant chromatogram was compared visually with a chromatogram of an Aroclor 1254 standard, but no quantitation was attempted.

Brady *et al.* (146) also reported the SFE of PCBs at about the same time on preparative scale equipment. A 10 g sample of soil contaminated with 1000 ppm of Aroclor 1254 was extracted with CO_2 at 103 kgf/cm^2 and 40 ^oC. It was reported that over 90 % of the PCBs were extracted in under one minute.

Onuska and Terry (147) also used SFE, coupled with GC-ECD, to look at a spiked river sediment sample containing PCBs, at approximately 2 mg/kg. In this paper, CO_2 was used as the supercritical fluid rather than the N_2O used by Hawthorne and Miller (145). Complete recovery of PCBs was reported, at 105.5 kgf/cm^2 and 60 ^oC, within 8 minutes. Experiments at 40 and 60 ^oC, and 105.5 and 211 kgf/cm^2 showed little difference between the two pressures, but slightly slower extraction at the higher temperature. The use of 2 % methanol as a modifier aided extraction, and the higher pressure setting was shown to give quantitative recoveries in a shorter time span. Quintuple repetitions of the extraction of different amounts of the sediment showed good accuracy and precision, when compared to the known concentrations. These were achieved at 211 kgf/cm^2 and 40 ^oC, with 2 % methanol as modifier.

Hawthorne *et al.* (148) used SFE with CO_2 to extract PCBs, and other compounds, from polyurethane foam sorbents. A pressure of 392.6 kgf/cm^2, and a temperature of 45 ^oC was used. The recovery of PCBs from the foam was investigated at two different spike levels, 1.5 mg and 1.5 μg, of Aroclor 1254. The extracts were collected in methylene chloride. Quantitative recoveries of all the different PCB congeners, by level of chlorination, were demonstrated for both levels of spike. Reproducability was shown by performing each extract three times. A GC-MS was used for analysis of the extracts.

Mulcahey *et al.* (149) used solid-phase extraction tubes as traps for the off-line SFE of PCBs from river sediments. The recoveries of five PCB congeners (PCBs No. 52, 101, 138, 170, and 180) were investigated using supercritical CO_2 at 361.6 kgf/cm^2 and 50 ^oC. The PCBs were spiked as a 2 μg/ml solution on to 2 g of river sediment. Recoveries, on a diol trap at -50 ^oC,

ranged from 44 - 65 %, although improved recoveries (45 - 78 %) were reported with 0.8 % toluene as a modifier.

Raymer and Velez (150) studied an on-line SFE-GC system for the recovery of PCBs, and other pollutants, from sea sand samples. The PCBs were spiked as a solution containing Aroclors 1232 and 1254 at either 17.1 μg/μl 1232 and 17.4 μg/μl 1254, or 688 ng/μl 1232 and 696 ng/μl 1254. Supercritical CO_2 at 413 kgf/cm^2 and 50 °C was used, and chromatograms obtained of both Aroclor spikes, which showed that all of the PCB congeners were present after a 7 minute extraction. The use of a Tenax-GC trap between the SFE and GC stages was also demonstrated. The presence of the trap was found to improve the chromatographic efficiency when compared to the result without the trapping step, under the same SFE conditions. The use of the trapping step also allowed larger volumes of extract from the SFE to be analysed by the GC, and the use of solvent modified CO_2 as the extraction fluid.

Hawthorne *et al.* (141) investigated three different supercritical fluids for the extraction of PCBs, and other pollutants, from a sediment sample. A standard sediment sample was obtained with known low μg/g concentrations of 9 individual PCB congeners. The majority of extractions were performed at 413 kgf/cm^2 and 50 °C for CO_2 and N_2O, or 100 °C for $CHClF_2$ (because of its higher critical temperature). To compare $CHClF_2$ and CO_2 extractions on a constant density and temperature basis, $CHClF_2$ extractions were also performed at pressures of 113.7 and 64 kgf/cm^2, to provide the same density as the 413 kgf/cm^2 CO_2 extractions, performed at 50 °C and 100 °C, respectively. In 40 minutes, the $CHClF_2$ gave quantitative recoveries of the 9 PCB congeners, whereas both CO_2 and N_2O gave an average of 62 % recovery for the congeners. A previous study by Onuska and Terry (147) led the authors to try CO_2 with 5 % methanol, as a modifier. Recoveries comparable to those obtained with $CHClF_2$ were obtained. Even at a pressure of 113.7 kgf/cm^2, $CHClF_2$ gave higher extraction efficiencies than CO_2 at 413 kgf/cm^2, and either 50 or 100 °C, suggesting that the polarity of $CHClF_2$, rather than the experimental fluid density or temperature, was responsible for the higher extraction efficiencies. Some losses of lower chlorinated PCB congeners during the extractions were reported.

Alexandrou *et al.* (151) investigated a number of different adsorbents for the possible fractionation of PCBs from polychlorinated dibenzo-p-dioxins (PCDDs) by SFE. Tenax, Florisil, alumina, carbon, and chemically modified silica (C_{18} and CN) were all used. Both supercritical CO_2 and N_2O were used, with all the different adsorbents, at 422 kgf/cm^2 and 40 ^oC, to extract a mixture of PCDDs and PCBs (8 - 455 ppb). Better recoveries were found throughout with N_2O than with CO_2. Florisil and alumina were found to be suitable adsorbents for this work, and Florisil was carefully investigated. It was found that a 15 minute extraction with CO_2 at 211 kgf/cm^2 and 40 ^oC removed over 75 % of the PCBs from Florisil, and full recoveries of the PCDDs were subsequently obtained by extracting with N_2O at 422 kgf/cm^2 and 40 ^oC for 90 minutes.

Van der Velde *et al.* (152) investigated the SFE of PCBs from soil. It was found that CO_2 at 204 kgf/cm^2 and 50 ^oC gave quantitative recoveries of PCBs No. 28, 52, 101, 118, 138, and 153, and a set of pesticides spiked at 5 ng/g, using a GC-ECD for analysis. Iso octane was found to be a better collection solvent than hexane. SFE was found to be more efficient than solvent extraction, and quicker than Soxhlet extraction for these compounds.

Langenfeld *et al.* (153) investigated the effects of pressure and temperature on the SFE extraction efficiency of PCBs, and PAHs, from river sediment. It was found that raising the extraction temperature from 50 ^oC to 200 ^oC greatly increased the SFE recoveries obtained using pure CO_2, while raising the extraction pressure from 361.6 to 671.6 kgf/cm^2, at conventional SFE temperatures (50 ^oC), had no effect on extraction efficiencies. These results indicated that reducing the kinetic limitations associated with overcoming the energy barrier of the desorption step, or possibly thermal alterations of the matrix, was needed to maximise SFE efficiencies, when analytes were tightly associated with sample matrices. The recoveries obtained with CO_2 at 200 ^oC compared favourably with those for $CHClF_2$, N_2O, and CO_2 with 5 % methanol found by Hawthorne *et al.* (141), thus possibly eliminating the need for less acceptable fluids, or the addition of modifiers.

7.1.3: INTRODUCTION TO SUPERCRITICAL FLUID CHROMATOGRAPHY:

The feasibility of using supercritical fluids for analytical chromatography was first demonstrated by Klesper *et al.* in 1962 (137). They demonstrated the enhanced solubility of some porphyrins in dichlorodifluoromethane and monochlorodifluoromethane, at pressures of up to 140.5 kgf/cm^2. Giddings *et al.* (154), in 1964, suggested the high separating potential of fluids at elevated pressures of about 1,033 kgf/cm^2. Throughout the 1960s, several research groups investigated the possible uses of SFC. Various fluids were used as the supercritical mobile phase, e.g. carbon dioxide, ammonia, sulphur dioxide, alcohols, chlorofluoromethanes, and low boiling point hydrocarbons. Most of this research used stainless steel packed columns, with instrumentation similar to that used for gas chromatography or HPLC at that time.

Although supercritical fluid chromatography (SFC) was introduced in the early 1960s, it was not until the early 1980s that the main breakthrough of SFC took place. This delay was caused by the technological difficulties in developing the proper instrumentation, and the fact that in the 1960s HPLC, rather than GC, was by far the most popular technique under investigation by the chromatographers of the day. The evolution of high-pressure technology in HPLC, and the advances in capillary-column technology in GC led to renewed interest in SFC from the early 1980s onwards (155).

It has been demonstrated that high chromatographic efficiency is possible, and that SFC has the ability to separate types of compounds which cannot be analysed by traditional gas chromatography. Rapid mass transfer in the supercritical mobile phase attracted researchers as it offered high-speed separation, with high resolution, on an open tubular capillary column (> 100 μm i.d.), which liquid chromatography cannot achieve, and also on packed capillary columns (> 1 mm i.d.) (156). The use of capillary columns has demonstrated the full potential of SFC as an analytical separation technique. Relatively non-volatile, thermally unstable, and high molecular weight solutes can all be analysed by SFC. For example, the analysis of thermally labile sulphonylureas by SFC has been reported by McNally and Wheeler (157). In addition, Sugiyama *et al.* (158) used SFC to investigate hydroperoxides in fats and oils.

Separations by SFC can be achieved and enhanced by varying the pressure, temperature, and mobile phase composition. The latter can be varied by the addition of organic solvents as modifiers, which change the eluting power of a supercritical mobile phase, such as carbon dioxide. A range of organic solvents, including methanol and acetonitrile, have been investigated as supercritical fluid modifiers by Levy and Ritchey (140).

7.1.3.1: Supercritical Fluid Chromatography Of PCBs:

There have been very few reports on the analysis of PCBs by SFC in the literature. Two papers on the SFC of PCBs both appeared in 1990. Onuska *et al.* (159) compared open-tubular and packed-column SFC separations of PCBs and terphenyls. They found packed-column SFC to be better suited than open-tubular column SFC for environmental monitoring, since the packed column was compatible with larger injection volumes and lower concentration-based detection limits.

Cammann and Kleiböhmer (160) investigated the retention characteristics of PCBs on cyanopropyl and octadecylsilane (ODS) columns, using carbon dioxide and nitrous oxide as eluents. A 20 cm x 1 mm i.d. ODS column, and a 25 cm x 1 mm i.d. cyanopropyl column were used. It was found that the ODS column showed greater retention of PCBs, and so a higher density (i.e. extraction pressure) was needed to elute PCBs from the ODS column as compared to the cyanopropyl column. It was also shown that higher chlorinated PCBs were retained longer on the ODS column than lower chlorinated PCBs, and that non-ortho-substituted PCB congeners (i.e. "planar" PCBs) were retained longer than ortho-substituted PCB congeners, within a group of chlorinated homologues, on the same ODS column. Similar results were obtained on the cyanopropyl column. The results demonstrated that the size and shape of the PCB isomers control retention in SFC, which agreed with results obtained for HPLC separations by de Kok *et al.* (161) and Bruggeman *et al.* (162). It was shown that increasing the temperature above the critical temperature resulted in lower retention times and, therefore, decreasing resolution. The best separations of a U.S. EPA mixture of PCBs No. 1, 5, 29, 50, 87, 154, 188, 199, and 209 on each

of the two different columns were shown. The rapid elution, within 16 minutes, of PCBs and PAHs in a sediment extract, using the cyanopropyl column, was shown. Carbon dioxide at 112 kgf/cm^2 and 80 ^oC was used.

7.1.4: INTRODUCTION TO COUPLED SFE-SFC:

The coupling of SFE to a chromatographic technique was first reported by Stahl (163), who developed a supercritical extraction system which was connected to a TLC, and investigated the extraction of natural products. Unger and Roumeliotis (164) reported a coupling device that allowed the on-line HPLC analysis of SFE extracts. They demonstrated the extraction of valtrate and didrovaltrate from *Radix valerianae* with carbon dioxide at 98 kgf/cm^2 and 40 ^oC, with subsequent HPLC analysis.

The coupling of SFE with SFC was first reported in 1985 by Sugiyama *et al.* (156), for the extraction and analysis of caffeine from coffee beans. The same research group produced several papers on the same subject at about this time. The system which was used incorporated 4 switching valves to allow the supercritical fluid to flow either through the extraction vessel, or through the chromatographic column. A trapping column, packed with activated carbon, was used to trap the extract prior to the chromatographic step. In 1986, Skelton *et al.* (165) demonstrated the use of SFE-SFC to analyse paprika. Both of these papers used packed-column SFC.

In 1987, Gmuer *et al.* (166) described a similar coupled SFE-SFC system, with a capillary column for the SFC step, and used it to analyse some natural products such as cheese, butter, coffee, tobacco, and camomile. McNally and Wheeler (157), in 1988, used a coupled system, with a switching device utilising 2 Rheodyne valves in series, to analyse sulphonylureas, their precursors, and metabolites from matrices such as soil, plant materials, and a cell culture medium.

The use of the cryogenic trapping of extracts prior to chromatography has been successfully achieved by a number of researchers. Engelhardt and Gross (167) showed the selective extraction of pesticides such as lindane, aldrin, and DDT from spiked soil (10 ppm of each pesticide), with cryogenic trapping. Thet used supercritical carbon dioxide at 141 kgf/cm^2 and 40 ^oC, with

stepwise extraction. A minimum detectable concentration of 1 ppm was reported. Raynor *et al.* (168) used an on-line SFE-capillary SFC system, incorporating cryogenic trapping, for the separation and identification of PAHs in coal pitch.

The use of 200 - 530 μm i.d. megabore capillary columns in the SFC stage has been reported by Hirata *et al.* (169), for analysing polyethylene film. An array of different detectors have been used, including FID, ECD, IR, and MS. Applications utilising SFE-SFC have been demonstrated in a number of papers, with cryotrapping as the most common collection method, but the use of sorbent adsorption has also been reported by some researchers. Two reviews covering the research efforts into coupled SFE-SFC have been written, by Vannoort *et al.* (170) and Greibrokk (171), respectively.

Vannoort *et al.* (170) reviewed the coupling of SFE with a range of chromatographic techniques such as TLC, HPLC, GC, and SFC. They found that SFE was more efficient, in terms of extraction times and recoveries, than Soxhlet extraction, and more suitable for thermolabile compounds. The reviewers concluded that efficient coupling was definitely possible, but that more work on the optimisation of experimental conditions was needed. The review contained 92 references.

Greibrokk (171) reviewed the coupling of SFE with HPLC, GC, and SFC, as well as the coupling of SFC with HPLC, GC, and SFC. The reviewer concluded that automated on-line multi-dimensional separation techniques reduced the analysis times of multi-component samples, and improved accuracy, reproducability, and detectability. The review contained 36 references.

7.1.4.1: The Use Of Coupled SFE-SFC For PCBs:

There has only been one report on the use of on-line SFE-SFC for the analysis of PCBs. Lin *et al.* (172) have recently reported the quantitative recovery of a mixture of Aroclors from an adsorbent resin using SFE-SFC. The recoveries of PCBs, PAHs, and OCPs from different adsorbents and soils were determined by SFE-SFC. A cryogenic trapping system was used between the SFE and SFC stages. A split injection technique was used, with the split off for 120

seconds to allow quantitative transfer of the SFE extract from the trap to the SFC column. A 10 m x 50 μm i.d. SB-Biphenyl-30 column, with a 0.25 μm film thickness, was used for the SFC stage, with an FID detector. Carbon dioxide was used for both stages, with pressure programming at 100 °C used to separate the PCBs by SFC. A 101.3 % recovery of a 1:1:1 mixture of Aroclors 1242, 1254, and 1260 spiked onto XAD-2 was reported, after a 20 minute extraction at 102 kgf/cm^2 (5 mins), and then 204 kgf/cm^2 (15 mins), with an extraction temperature of 60 °C. A pressure programme of 74 kgf/cm^2 for 5 mins, followed by a ramp of 4 kgf/cm^2 per minute to 255 kgf/cm^2 was used for the SFC stage. Recoveries of the individual Aroclors from XAD-2 and Tenax-GC adsorbents, and two soil matrices were between 58 % and 103 %, for a 7 minute extraction at 255 kgf/cm^2 and 60 °C, with the same SFC conditions as previously outlined. The XAD-2 adsorbent showed lower recoveries because it has a large surface area and small pore size, making extraction more difficult. A range of PAHs and OCPs were also quantitatively extracted with the same SFE-SFC equipment.

7.1.5: THE USE OF COUPLED SFE-GC FOR PCBS:

The technique of coupled SFE-GC has been used by a number of researchers to analyse PCBs. The first report of the direct coupling of SFE with GC was published by Hawthorne and Miller (143). The supercritical fluid extraction of a Tenax-GC sorbent trap, with cryogenic trapping of the extracted organics in a gas chromatographic column, was demonstrated by the analysis of an automobile exhaust sample collected on the Tenax-GC trap. Supercritical CO_2, at 207 kgf/cm^2 and 45 °C, was used to extract the organic species from the exhaust sample for 10 minutes. The end of the capillary restrictor from the SFE equipment, used to maintain the pressure at 207 kgf/cm^2, was inserted directly into the gas chromatographic column through the on-column injection port, which was kept at 0 °C during the 10 minute extraction period. After the extraction, the capillary restrictor was pulled out of the injection port and the CO_2 was allowed to flush out of the GC column for 1 minute. The GC was then rapidly heated to 50 °C, and the extract analysed on a 40 m DB-5 column with an FID, showing acceptable chromatographic peak shapes.

The same researchers also reported the use of directly coupled SFE-GC for the analysis of PCBs and PAHs from sediment, and other environmental matrices (145). The use of FID, ECD, and MS detectors was reported, and the experimental conditions needed for each were investigated. SFE-GC-ECD was used to analyse 10 mg samples of river sediment that had been spiked with 8.0 μg/g of Aroclor 1254. The river sediment sample was extracted for 10 minutes with nitrous oxide at 310 kgf/cm^2 and 45 °C. After the extraction, the chromatographic column (30 m x 0.32 mm i.d., 1 μm film thickness, DB-5) was flushed with carrier gas for 2 - 3 minutes, while the GC oven temperature was held at the 5 °C trapping temperature. This procedure allowed the nitrous oxide to be removed from the chromatographic column, since nitrous oxide gives a relatively high ECD response. After the flushing step, the GC oven was rapidly heated to 200 °C, followed by temperature programming at 8 °C/min to 320 °C. The results obtained were compared with those for standard Aroclor 1254, and it was shown that all the PCB congeners in Aroclor 1254 had been recovered, and good chromatographic peak shapes were also achieved. More results for a range of other matrices using supercritical CO_2 or N_2O, for the analysis of a variety of organic compounds, have subsequently been reported by the same researchers, e.g. (148) and (173).

The practical problems of using capillary restrictors, and the problems of getting rid of the CO_2 or N_2O led Nielen *et al.* (174) to use a different design to couple SFE with GC, so that it could be used to analyse compounds in environmental samples at low levels. The SFE-GC system of Nielen *et al.* used a cold trap before the GC injector to collect the extract, which could then be swept on to the GC column by the use of switching valves. 100 mg of Tenax-GC was spiked with 10 μl of an acetone solution containing 1,500 pg of hexachlorobenzene (HCB) and 300 pg each of PCB No. 101, 153, and 180. This was extracted with CO_2 at 204 kgf/cm^2 and 42 °C, and collected on the cold trap at 5 °C. The cold trap was flash-heated to 300 °C, and the released components transferred to the capillary GC column. The extract was analysed on a 60 m x 0.22 mm i.d. DB-1 column with an ECD detector. Recoveries of between 50 and 65 % were achieved, with a detection limit of about 30 pg for the individual PCBs. Impurities in the carbon dioxide were found to hamper the detection of PCB levels lower than 30 pg.

Onuska and Terry (147) also used a trap in their SFE-GC system, and analysed spiked river sediment for PCBs using an ECD detector. CO_2 at 211 kgf/cm^2 and 40 ^oC was used, with 2 % methanol as modifer, to achieve the highest recovery. The trap was held at 5 ^oC during extraction, and expanded mobile phase in the gaseous state was vented to the atmosphere. This meant that no methanol got onto the analytical GC column (30 m x 0.25 mm i.d. SE-52). Reproducible, quantitative recoveries of PCBs from spiked river sediment, down to about 20 ng, were achieved.

Raymer and Velez (150) looked at the use of a Tenax-GC cartridge as a trap for the extraction of PCBs and pesticides from sea sand. CO_2 at 413 kgf/cm^2 and 50 ^oC was used for the extraction stage. The Tenax-GC cartridge was found to be useful as a trap, with no great loss of chromatographic resolution. Aroclors 1232 and 1254 at about 17 μg/μl and 700 ng/μl were used as spikes, and all the PCB homologues were recovered. This system allowed the use of methanol as a modifier for the SFE phase.

7.1.6: THE USE OF STATISTICAL OPTIMISATION FOR WORK WITH SUPERCRITICAL FLUIDS:

A statistical approach to try to optimise SFE was first attempted by Lopez-Avila *et al.* (175), who used a partial factorial design method. Seven variables were studied and only eight experiments were performed, 1/16th of the full design. It was concluded that the statistical significance of the seven variables could not be properly tested from just eight experiments.

Ho and Tang (176) used a full factorial design to evaluate the relative importance of extraction pressure, temperature, and the length of extraction time, on the efficiency of SFE for extracting organic pollutants from a liquid-solid extraction cartridge, used to isolate organic pollutants from water. The recoveries of 29 PAHs and pesticides were studied. A statistical analysis of variance (ANOVA) was performed to evaluate the effects of the experimental variables. Pressure was the most influential variable, affecting the recoveries of all the compounds studied. The higher the pressure, the higher the recoveries. The next most influential factor, extraction

time, affected the recoveries of some of the compounds. The temperature affected the recoveries of only a few compounds. Interactions of the three variables also affected extraction efficiencies.

A variable-size simplex method has been proposed by Morgan and Deming (177) to optimise an SFE system. A geometric figure of the simplex is defined by a number of points (n + 1) equal to one more than the number of variables under investigation (n). In this case, the initial study of three variables comprised four experiments, each at a different set of experimental conditions. The measured response was percentage recovery. The results were represented in three-dimensional space (three variables) as the vertices of a tetrahedron. The set of experimental conditions giving the lowest response (recovery) was rejected, and another experiment carried out at a set of conditions determined statistically. This result, along with the three remaining results from the initial experiments, formed a new tetrahedron. The set of conditions giving the lowest response was, again, rejected and another experiment conducted. This procedure was continued until all the coordinates of a tetrahedron gave similar, high responses. The simplex was then stopped. The optimum conditions for pressure, temperature, and extraction time fell somewhere within this final tetrahedron.

The factorial design helped to choose the initial conditions of the simplex operation. Then the simplex method simultaneously searched for optimum levels of the three factors of interest (pressure, temperature, and extraction time). It was found that extraction conditions of 361.6 kgf/cm^2 to 413 kgf/cm^2, a temperature of about 40 ^oC, and an extraction duration of between 20 and 35 minutes, gave quantitative recoveries of most of the analytes. The addition of methanol as a modifier enhanced the recoveries of those compounds which were not quantitatively extracted, under the optimum set of conditions, with pure supercritical CO_2.

Bicking *et al.* (178) used an experimental design strategy to optimise the temperature and pressure conditions for the SFE of hydrocarbons from soil samples. Diatomaceous earth was spiked with hexadecane, and extracted with supercritical CO_2, using nine different temperature and pressure combinations. The percentage recovery of the hexadecane was determined for each experiment. Linear regression of the recovery data allowed the construction of a percentage

recovery response surface. Comparison of SFE at near to the calculated optimum temperature and pressure values, 55 ^{o}C and 300 kgf/cm^2, with Soxhlet extraction and solvent shaking showed good agreement of percentage recoveries between the three methods. The Soxhlet and shaking methods were U.S. EPA methods, and both employed Freon solvents. These are environmentally damaging, and alternatives to their use are being sought. Bicking *et al.* have shown the efficacy of the use of SFE as an alternative to the existing EPA extraction methods.

Oostdyk *et al.* (179) optimised the SFE of primary aromatic amines from topsoil with nitrous oxide. Extraction pressure, modifier, temperature, and extraction time were investigated separately. The optimised conditions were found to use nitrous oxide at 361.6 kgf/cm^2 and 50 ^{o}C, with 5 % 1,6-hexanediamine in methanol as the modifier.

The effects of pressure, temperature, and modifier on the SFE of organochlorine and organophosphate pesticides from different soil matrices were investigated by Snyder *et al.* (180). The use of CO_2, with 3 % methanol as modifier, gave better recoveries of the pesticides than CO_2 alone. Recoveries increased with increasing pressure up to a threshold, about 361.6 kgf/cm^2, above which maximum recoveries of the pesticides were obtained. Temperature was found to have little effect on recoveries over a range of 40 ^{o}C to 120 ^{o}C. The presence of a small amount of moisture was found to increase recoveries, while acidic or basic conditions reduced the recoveries of the pesticides.

7.2: MATERIALS AND METHODS:

7.2.1: SUPERCRITICAL FLUID EXTRACTION (SFE):

SFE conditions were investigated and optimised for the extraction of PCBs from a matrix of freeze-dried milk and Florisil, using supercritical carbon dioxide. The extraction of PCBs from freeze-dried milk was previously carried out in the laboratory by Soxhlet extraction, followed by a step to reduce the solvent volume. This process took many hours to perform, and it was hoped, therefore, that SFE would speed up the extraction time considerably.

Next, the SFE of fat from freeze-dried milk using supercritical CO_2 was investigated, using the same optimisation procedure as for the extraction of PCBs. This gave a direct comparison of the conditions needed to extract PCBs and fat, from which it could be easily seen whether a further step to separate the PCBs from fat was needed, before the final chromatographic analysis of an extract was performed.

7.2.1.1: SFE Experimental:

A Jasco Supercritical Fluid system was kindly donated by Mettler-Toledo Ltd. (Halstead, G.B.). A schematic of the supercritical fluid apparatus can be seen in Diagram 7.2.

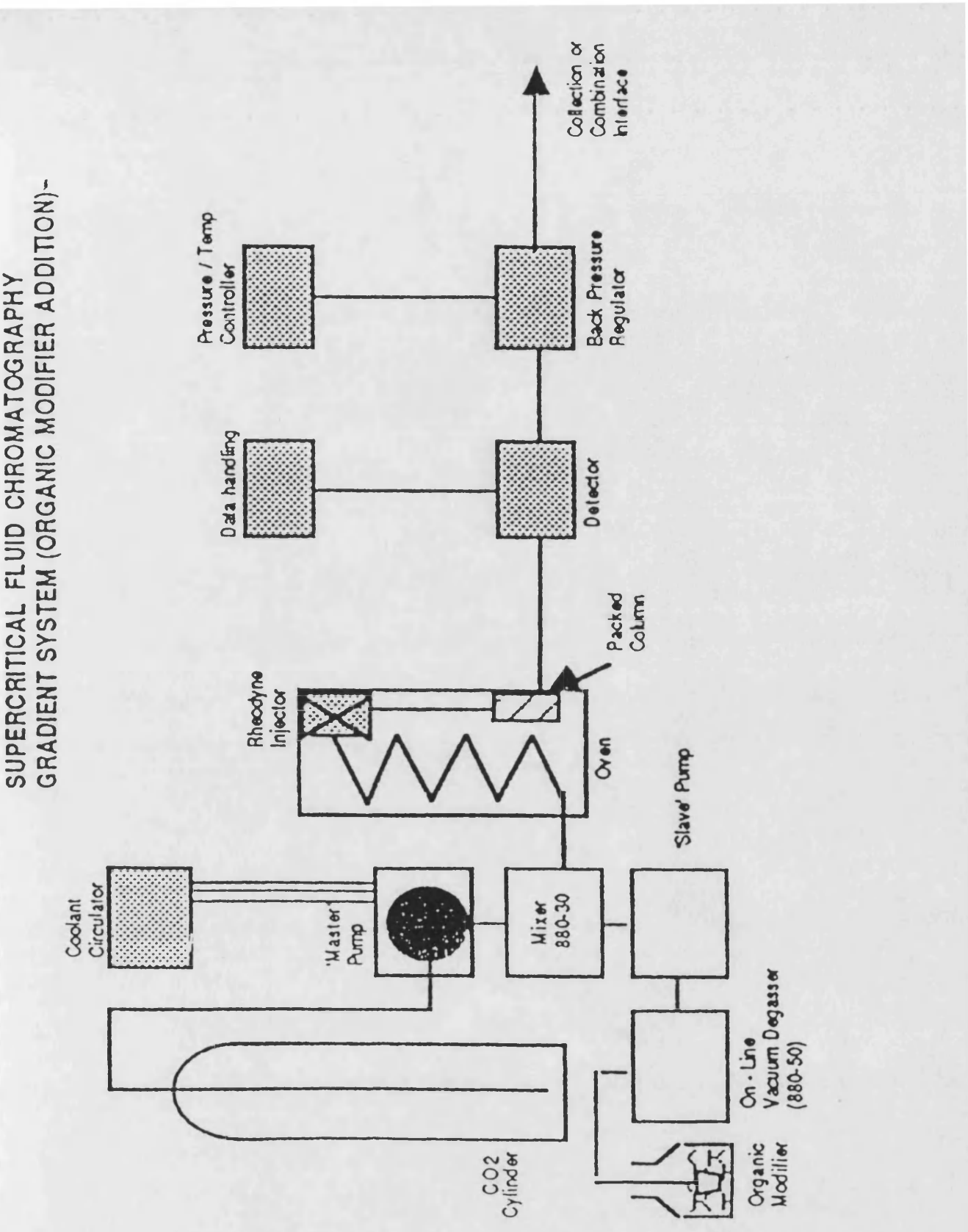


DIAGRAM 7.2: SUPERCRITICAL FLUID APPARATUS FROM METTLER-TOLEDO LTD.

An ethylene glycol filled cooler was used to maintain the head of an 880-PU HPLC pump at -10 °C. The ethylene glycol was pumped through a jacket surrounding the head of the HPLC pump. This HPLC pump was used for pumping liquid carbon dioxide from a cylinder (BOC, Guildford, G.B.). The flowrate of the CO_2 was controlled using the electronic keypad of the HPLC pump. The extraction vessel, consisting of an empty 13 cm x 10 mm i.d. HPLC column with two screw-capped ends, was housed in a model 860-CO column oven. The column oven had a built-in temperature controller. A heat exchange coil, situated inside the column oven before the extraction vessel, ensured that the CO_2 reached equilibrium before entering the extraction vessel. The material to be extracted was packed inside the HPLC column, and the two ends screwed on tightly by hand.

A Rheodyne switching valve was used to switch the flow of supercritical CO_2 through the extraction vessel, once a sample had been loaded. A model 875-UV ultraviolet detector, with a specially manufactured high pressure flow cell, was used to continuously monitor the extract at a specific wavelength. A chart recorder was used to trace the UV absorbance throughout an extraction. A model 880-81 back pressure regulator kept the entire extraction system at a specified constant back pressure, via an electronic feedback regulator that was flow independent. This arrangement provided greater stability throughout the system than the more frequently used capillary restrictors. The temperature of the back pressure regulator was also controlled to avoid the problem of the extracts becoming plugged in the exit tubing. The extracts were vented to the atmosphere through the back pressure regulator, and collected in an organic solvent in ice-cooled test tubes.

The CO_2 flowed from the cylinder to the HPLC pump as a liquid. The cooling of the head of the HPLC pump meant that the CO_2 remained liquid upon entering the pump. The liquid CO_2 was then pumped at a specified pressure, maintained by the back pressure regulator, and flowrate to the column oven. Before entering the column oven the CO_2 could be said to be in the subcritical state. This means that the CO_2 was above its critical pressure (75.2 kgf/cm^2), but below its critical temperature. Upon entering the column oven the CO_2 became supercritical because the

temperature of the oven was set above the critical temperature of CO_2 of 31 ^oC. The heat exchange coil gave the CO_2 time to equilibrate before entering the extraction vessel. The CO_2 remained supercritical until vented to the atmosphere after the back pressure regulator, at which point it became gaseous, its normal state under ordinary laboratory conditions. The end of the exit tubing from the back pressure regulator was submerged in the cooled collection solvent, so that the now gaseous CO_2 bubbled out through the solvent. The extracted compounds were left behind dissolved in the collection solvent.

A known weight of freeze-dried skimmed milk, equivalent to 10 ml of the original milk before freeze drying, was used for each extraction. This quantity of freeze-dried milk was the same as was used for other work reported in Chapter 2. The low level of fat in skimmed milk (fat content = 0.1 %) ensured that the interference of the UV signal, monitored for extracted PCBs, by the milk fat was minimised. Florisil (Aldrich Chem. Co. Ltd., Gillingham, G.B.) was added to the freeze-dried milk to aid the rapid penetration of the milk by the supercritical CO_2.

For each extraction, the freeze-dried milk was mixed with enough Florisil to ensure that the extraction vessel would be full. The mixture was ground together using a mortar and pestle, and poured into the extraction vessel through a glass funnel. A known volume of the Aroclor 1242 spike, which was used throughout the SFE experiments, was carefully added using a syringe. The solvent from the spiking solution was allowed to evaporate before the top of the extraction vessel was screwed on by hand. The extraction vessel was then attached to the Rheodyne valve in the column oven.

The extraction vessel was connected to the six-port Rheodyne valve in the same way as an injection loop would ordinarily be attached for HPLC work. This meant that with the valve in the "load" position, the extraction vessel was completely bypassed. Therefore, the vessel could be taken out of the column oven for filling and/or emptying without disturbing the flow of the supercritical CO_2. After replacing the vessel in the oven, the Rheodyne valve could be turned to the "inject" position, allowing supercritical CO_2 to flow through the extraction vessel.

The required extraction pressure was set on the back pressure regulator, and the extraction temperature was set on the column oven. Once the whole system had stabilised under the set conditions, a steady flow of supercritical fluid exited from the back pressure regulator. This was signified by the movement of the needle valve in the back pressure regulator, causing a clearly audible purring sound. The needle valve opened and closed automatically to keep the back pressure constant, and the speed of this motion was manually controlled from a screw valve on the top of the back pressure regulator. Manual adjustment was carried out until the variation of the back pressure from the set value was at a minimum, at which time the purring sound was emitted.

An extraction was initiated by turning the Rheodyne valve quickly from the load position to the inject position. The pressure in the system immediately fell as the supercritical fluid entered, and began to fill, the extraction vessel. During this period no supercritical fluid was emerging from the back pressure regulator. The pressure, as read from the back pressure regulator, gradually built back up as the vessel filled. Once the back pressure had re-reached the set value, the CO_2, with the extracted compounds, began to emerge from the back pressure regulator, which emitted a purring sound. The extracts were then collected in a cooled solvent, usually HPLC grade heptane (Fisons Sci. Equip., Loughborough, G.B.), as previously described.

All of the extracts collected during this piece of work were analysed by GC-MS and/or GC-ECD. Injections of a standard Aroclor 1242 solution were made on both instruments. These standard injections were compared with the extract injections to see exactly what each extract contained. Temperature programmes already in routine use in the laboratory were used on both the GC-MS and the GC-ECD to analyse the extracts for PCBs. An arithmetical method reported by Erickson *et al.* (69) was used to positively identify peaks on the GC-MS, whereas retention times were used for peak identification on the GC-ECD.

7.2.1.2: Gas Chromatography-Mass Spectrometry (GC-MS):

All of the collected samples were analysed by an HP 5890 gas chromatograph with a 5970 MSD mass spectrometer and a 7673A automatic sampler (Hewlett-Packard, Bracknell, G.B.). A 50 m x 0.2 mm i.d. HP-1 capillary column (Hewlett-Packard, Bracknell, G.B.), with a film thickness of 0.33 μm, was used for the analyses. A selected ion monitoring (SIM) programme designed specifically for the analysis of PCBs was used throughout. A 5 μl aliquot of each sample was injected onto the column, with the injector in the splitless mode. The injector temperature was set at 250 ^{o}C, and the volumetric flowrate of the hydrogen carrier gas was set at 1 ml/min. A temperature programme, previously developed in the laboratory, was used to separate as fully as possible all the individual PCB congeners present in the samples. The temperature programme consisted of an initial temperature of 75 ^{o}C held for 2 minutes, followed by a ramp of 30 ^{o}C/min up to 120 ^{o}C, then a ramp of 10 ^{o}C/min up to 270 ^{o}C. The final temperature of 270 ^{o}C was held for 35 minutes. The SIM programme was designed so that at any given time during a chromatographic run, the MSD was monitoring for 4 mass ions specific to two different levels of PCB chlorination, Chapter 2.

Every peak found by the GC-MS was mathematically tested to decide whether or not the peak was due to a PCB congener. The method of Erickson *et al.* (69) was adapted for this purpose. The peaks that failed this mathematical test were ignored, and only those peaks that passed were used to assess the levels of PCBs in the samples.

7.2.1.3: Gas Chromatography-Electron Capture Detection (GC-ECD):

An 8320B capillary GC-ECD (Perkin-Elmer, Beaconsfield, G.B.) was used to analyse some of the collected samples. A 25 m x 0.22 mm i.d. HT-5 column (SGE Pty. Ltd., Milton Keynes, G.B.), with a film thickness of 0.1 μm, was used for the analyses. The injector and detector temperatures were set at 250 ^{o}C, and hydrogen, set at 6 p.s.i., was used as the carrier gas, with nitrogen as the make-up gas. A ^{63}Ni electron capture detector was used. A temperature programme was developed for the analyses. The temperature programme consisted of an initial

temperature of 75 °C held for 2 minutes, followed by a ramp of 30 °C/min up to 120 °C, then a ramp of 1.5 °C/min up to 210 °C. The final temperature of 210 °C was held for 10 minutes.

The chromatograms of each sample were printed out on a GP-100 graphics printer (Perkin-Elmer, Beaconsfield, G.B.). The identities of the various peaks were found by comparison of the retention times with those of a chromatogram of an Aroclor 1242 standard.

7.2.2: SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC):

SFC was investigated for the separation of fat from PCBs. Previously, this separation step has been performed using an HPLC method developed in this laboratory (56). It was hoped that SFC would also prove capable of separating the fat from the PCBs. SFC has the advantage over HPLC that, even with modifier present, it uses less organic solvent than HPLC. This is advantageous environmentally, and also avoids the need to greatly reduce the volume of the final extract, before its analysis by GC.

The behaviour of Aroclor 1242 under various SFC conditions, using different columns, was investigated. Then a sample of fat was injected under the same conditions. Finally, a mixture of fat and PCBs was injected to ascertain whether a separation could be achieved.

7.2.2.1: SFC Experimental:

The same supercritical fluid system, kindly donated by Mettler-Toledo Ltd. (Halstead, G.B.), was used for the SFC work as for the SFE work with a few minor alterations, Diagram 7.2. A second model 880-PU HPLC pump was used to allow the addition of an organic modifier to the supercritical CO_2 mobile phase. The organic modifier used throughout this work was HPLC grade 2-propanol (Fisons Sci. Equip., Loughborough, G.B.). The two mobile phase components were mixed in a model 880-30 mixer module, prior to entering the 860-CO column oven.

Three different HPLC columns were investigated during this work. The columns used were a 30 cm x 7.5 mm i.d. Polymer PLRP-S column (Polymer Labs., Church Stretton, G.B.), a 10 cm x

2.1 mm i.d. Brownlee Labs. RP-8 Spheri-5 column (Anachem, Luton, G.B.), and a 15 cm x 4.1 mm i.d. Hamilton PRP-1 column (Jones Chromatogr., Hengoed, G.B.).

Two Rheodyne valves in series were required to set up the system for the SFC work. The first Rheodyne valve allowed a 20 µl injection loop to be used to introduce the samples onto the column. The HPLC column was attached to the second Rheodyne valve. This arrangement allowed samples to be injected into the injection loop with the Rheodyne valve in the load position. While this was done, there was no flow of mobile phase, or consequent build-up of pressure, in the loop. In other words, the injection loop could be isolated from the rest of the system. Once the sample had been loaded into the injection loop, it was introduced onto the column by turning the first Rheodyne valve from the load position to the inject position. This allowed the mobile phase to flow through the injection loop and on into the column. By turning the valve back to the load position, the flow bypassed the injection loop, and the pressure in the loop was released, ready for the next injection to be made.

The eluents were collected in the same way as for the SFE work. The UV detector was used at a wavelength of 254 nm, and a range of 0.08 a.u.f.s. A small volume of heptane in ice cooled test tubes was used to collect the extracts once the back pressure in the system had reached its set value, after an injection had been made. It was noted that the HPLC columns utilised in this work should not be used at pressures greater than 3000 p.s.i. (= 211 kgf/cm^2).

All of the fractions collected in the SFC work were analysed by GC-MS and/or GC-ECD. Exactly the same temperature programmes were used as described for the SFE work. Injections of a standard Aroclor 1242 solution were again used to find out exactly what each collected fraction contained. All of the relevant chromatographic parameters are given in the SFE materials and methods, Sections 7.2.1.2 and 7.2.1.3.

7.2.3: COUPLED SFE-SFC WORK:

The combining of the SFE step with the SFC step would provide a system where a fatty food sample, which was to be analysed, could be put in an extraction vessel at the beginning, and a

solution, containing the compounds of interest, could be collected at the far end of the chromatography column. This solution would be ready for analysis by GC without further manipulation.

Such a system would greatly increase the number of samples that a laboratory could perform at any one time, because of the much shorter experimental times of the extraction and chromatographic steps compared to traditional methods. The use of organic solvents would be minimised, saving money, and cutting out the need for the disposal of large quantities of waste solvents. The number of sample manipulation steps would be reduced, thus lessening the risks of sample loss or contamination.

Some initial experiments to construct and successfully use a coupled SFE-SFC system are reported. A trapping column was used between the SFE and SFC steps to trap the SFE extract off-line, before it was chromatographed on the SFC system.

7.2.3.1: Coupled SFE-SFC Experimental:

A directly coupled SFE-SFC system was designed and constructed. It used 4 Rheodyne valves, so that the SFE step could be carried out, and the extract trapped on a trapping column. Then the Rheodyne valves were switched, so that the extract could be swept from the trapping column to the chromatography column. The final eluent was collected in a cooled solvent, as in the previous work. The same extraction vessel and chromatographic column were used as previously reported in Sections 7.2.1 and 7.2.2.

The SFE step was carried out exactly as in Section 7.2.1.1. The extract passed out of the back pressure regulator and onto the trapping column. The far end of the trapping column was open to the atmosphere, so that the pressure in the trapping column was much lower than that set for the SFE system. This lower pressure in the trapping column, along with the fact that the column was cooled in ice, would hopefully cause the extracted compounds from the SFE step to be trapped on the column, and not escape to the atmosphere.

Once the SFE stage had been completed, the trapping column was immersed in a water bath, at the same temperature as the column oven, and the positions of the four Rheodyne valves were changed. The supercritical fluid then flowed through the trapping column, hopefully sweeping the SFE extract onto the chromatographic column. The final eluent was collected in an organic solvent in ice-cooled test tubes.

7.3: RESULTS:

7.3.1: RESULTS FOR SFE WORK:

7.3.1.1: SFE Of PCBs From Cows Milk:

Some initial experiments were carried out to determine the correct concentration of the PCB spike needed to obtain a reasonable response on the chart recorder, which was attached to the UV detector. Aroclor 1242 was used at differing concentrations, using peak heights as the measured response. Peak areas gave inaccurate estimates of extraction efficiency due to the fluctuating nature of the recorded baseline. The highest possible percentage extraction, in the shortest possible time, was the optimum result that was required.

Throughout the optimisation of the extraction pressure and temperature, the liquid CO_2 flowrate from the HPLC pump was set at 3.0 ml/min, and the UV detector was used at a wavelength of 254 nm, and a range of 0.08 aufs. Initially, moderate extraction conditions of 200 kgf/cm^2 and 50 ^oC were used, and the concentration of PCBs added, as Aroclor 1242, was increased gradually, until a suitable response was found on the chart recorder. A peak for the Aroclor 1242 spike was eventually observed on the chart recorder at between 5 and 10 minutes. After a number of experiments, a 50 µl spike of 100 ppm Aroclor 1242 was found to give the required peak height response, and this level of spike was used throughout the optimisation procedure.

Once the correct concentration of the spike of Aroclor 1242 had been determined, a Simplex optimisation procedure, first described by Yarbro and Deming (181), was undertaken. The extraction temperature and back pressure were selected, by referring to the work of other researchers, as the significant factors of the extraction which were to be optimised. This was because the solvent strength of a supercritical fluid is determined by its density, as well as a number of other factors. Therefore, the solvating ability of a particular supercritical fluid, i.e. CO_2, towards a particular species, i.e. PCBs, can be modified by changing the extraction pressure, and also the temperature. These two parameters were varied by the method described by Yarbro and Deming (181), using mathematical formulae defined by Miller and Miller (182).

A simplex is a geometrical figure which has (n + 1) vertices, each of which is located by n coordinates, when a response is being optimised with respect to n factors. For example, in this case, for two factors the simplex would be a triangle. The optimisation begins by defining the initial simplex triangle, and continues by calculation of the vertices. The initial triangle is set up by measuring a response, e.g. peak height, peak area, UV absorbance, at three different combinations of levels of the two significant factors. The three sets of conditions for initial investigation are chosen so that they are distant from the expected optimal conditions. The extracting strength of supercritical CO_2 usually increases with increasing pressure, and usually decreases with increasing temperature. Therefore, the optimisation procedure was started at a high extraction temperature and a low back pressure, because this was expected to give a poor extraction efficiency of PCBs from cows milk. Of course solvation is not the only limiting process of SFE with CO_2, matrix interactions are also an important consideration (153).

The area of the pressure:temperature surface in which the initial simplex triangle should be situated was investigated. The extraction of a spike of 50 µl of 100 ppm Aroclor 1242, from a mixture of freeze-dried skimmed milk and Florisil, was carried out at 120 kgf/cm^2 and 65 ^{o}C. Once the mixture for extraction had been loaded into the extraction vessel, the vessel was attached to the Rheodyne valve. The Rheodyne valve was turned to the inject position and, once the back pressure had again reached 120 kgf/cm^2, the extract was collected for a known period of time in

ice-cooled heptane. Two fractions of the extract were collected in separate test tubes, the first between 0 and 15 minutes, and the second between 15 and 30 minutes, after the extraction began. Only a small peak for the extraction of PCBs, expected at between 5 and 10 minutes, was observed. However, the subsequent analysis of the two extract fractions by GC-MS showed the presence of the typical Aroclor 1242 pattern in both of the fractions. This meant that this set of extraction conditions did extract Aroclor 1242 from the milk and Florisil matrix, but that the extraction took at least 15 minutes and, possibly, was not even complete after 30 minutes. Another extraction was done at 120 kgf/cm^2 and 55 ^{o}C, meaning a higher density of the CO_2, which gave a similar result to the first extraction.

When conditions of 160 kgf/cm^2 and 55 ^{o}C were selected, a peak was obtained on the chart recorder, Diagram 7.3a.

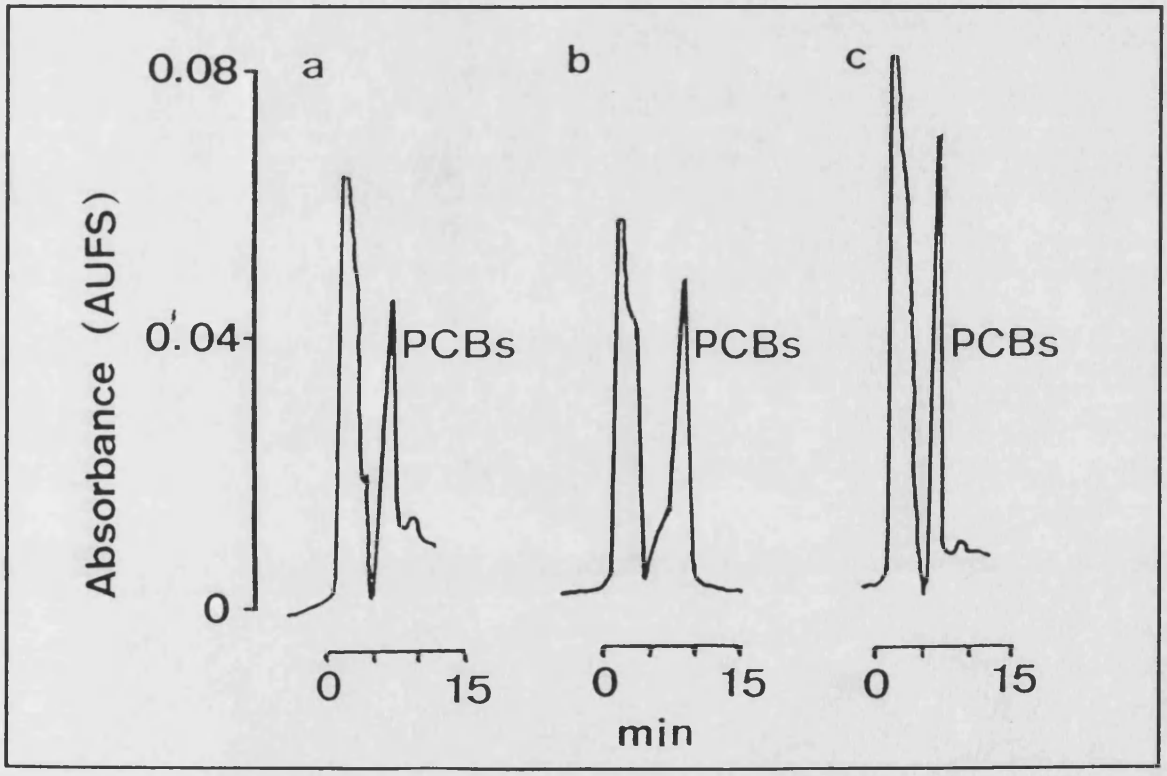


DIAGRAM 7.3: THE SFE OF PCBS FROM FREEZE-DRIED, SKIMMED MILK WITH A SPIKE OF 50 µl OF 100 ppm AROCLOR 1242

a. 55 ^{o}C:160 kgf/cm^2 b. 45 ^{o}C:240 kgf/cm^2 c. 47 ^{o}C/220 kgf/cm^2

The first triangle of the simplex optimisation procedure was then set up by measuring the peak height response, in duplicate, under this set of conditions (point 3), at 120 kgf/cm^2 and 65 ^{o}C (point 1), and at 120 kgf/cm^2 and 55 ^{o}C (point 2). This can be seen in Diagram 7.4.

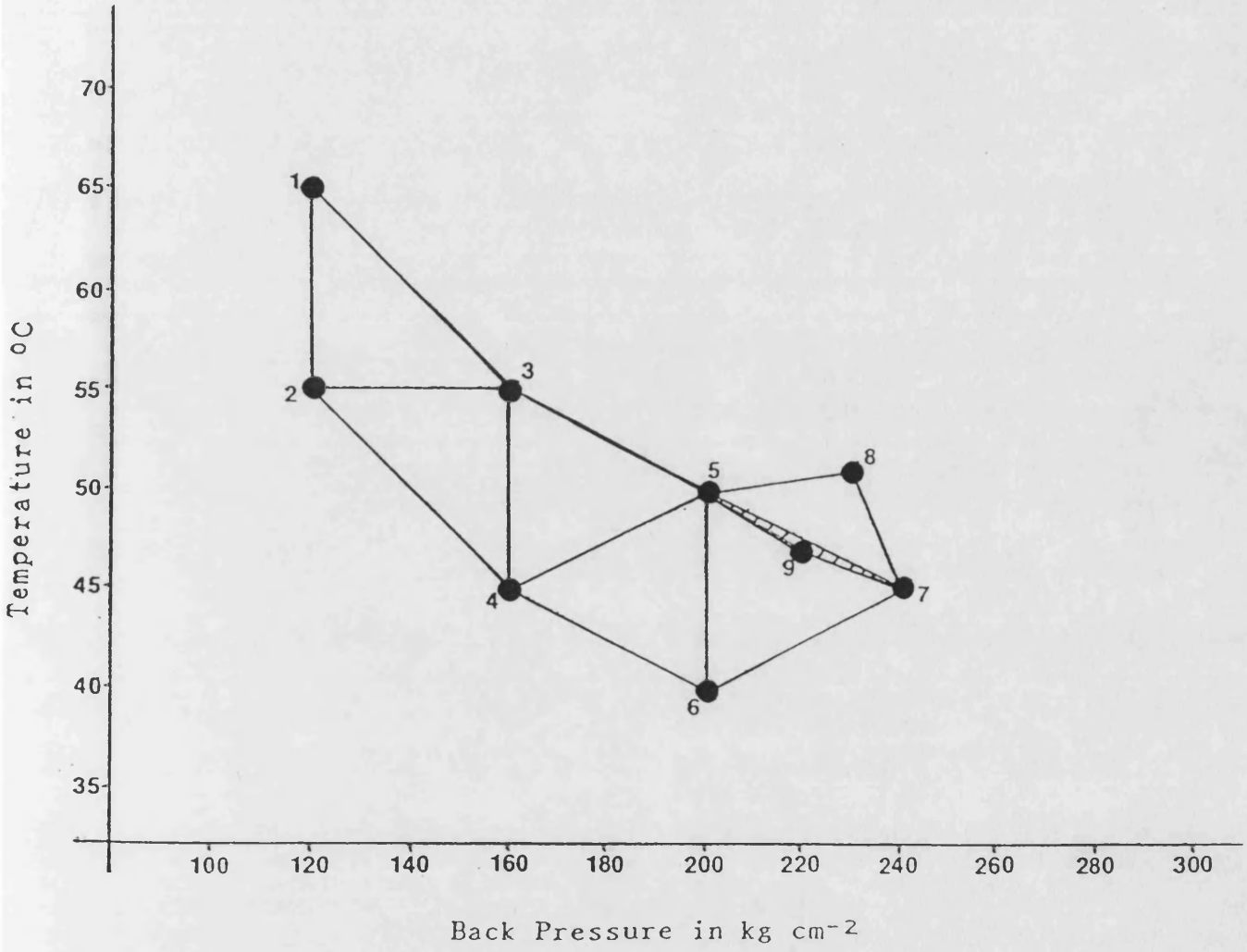


DIAGRAM 7.4: SIMPLEX OPTIMISATION PROCEDURE FOR THE EXTRACTION OF PCBS FROM FREEZE-DRIED, SKIMMED MILK

The set of conditions giving the smallest peak height response, in this case 120 kgf/cm^2 and 65 ^oC, was rejected and the next set of experimental conditions was calculated according to Miller and Miller (182).

An extraction pressure of 160 kgf/cm^2 and temperature of 45 ^oC was used for the next group of extractions (point 4). Duplicate extractions of the same level of PCB spike from freeze-dried milk and Florisil were performed, and the average peak height response was calculated. A new simplex triangle was drawn, and again the set of conditions giving the lowest peak height response, here 120 kgf/cm^2 and 55 ^oC (point 2), was rejected. The simplex optimisation process was continued until a triangle had been constructed where each of the three sets of conditions gave a similar peak height response. This final triangle was made up of points 5, 7, and 9, and is shaded in Diagram 7.4. The optimum extraction conditions for the extraction of PCBs from cows milk, using supercritical CO_2, lie within this final triangle. The last point to be investigated (point 9), 220 kgf/cm^2 and 47 ^oC, achieved an extraction of Aroclor 1242 from cows milk in approximately 10 minutes (Diagram 7.3c).

All of the extracts from the simplex optimisation procedure were collected and analysed by GC-MS, and all of the extracts showed the characteristic pattern for Aroclcr 1242, Diagram 7.5.

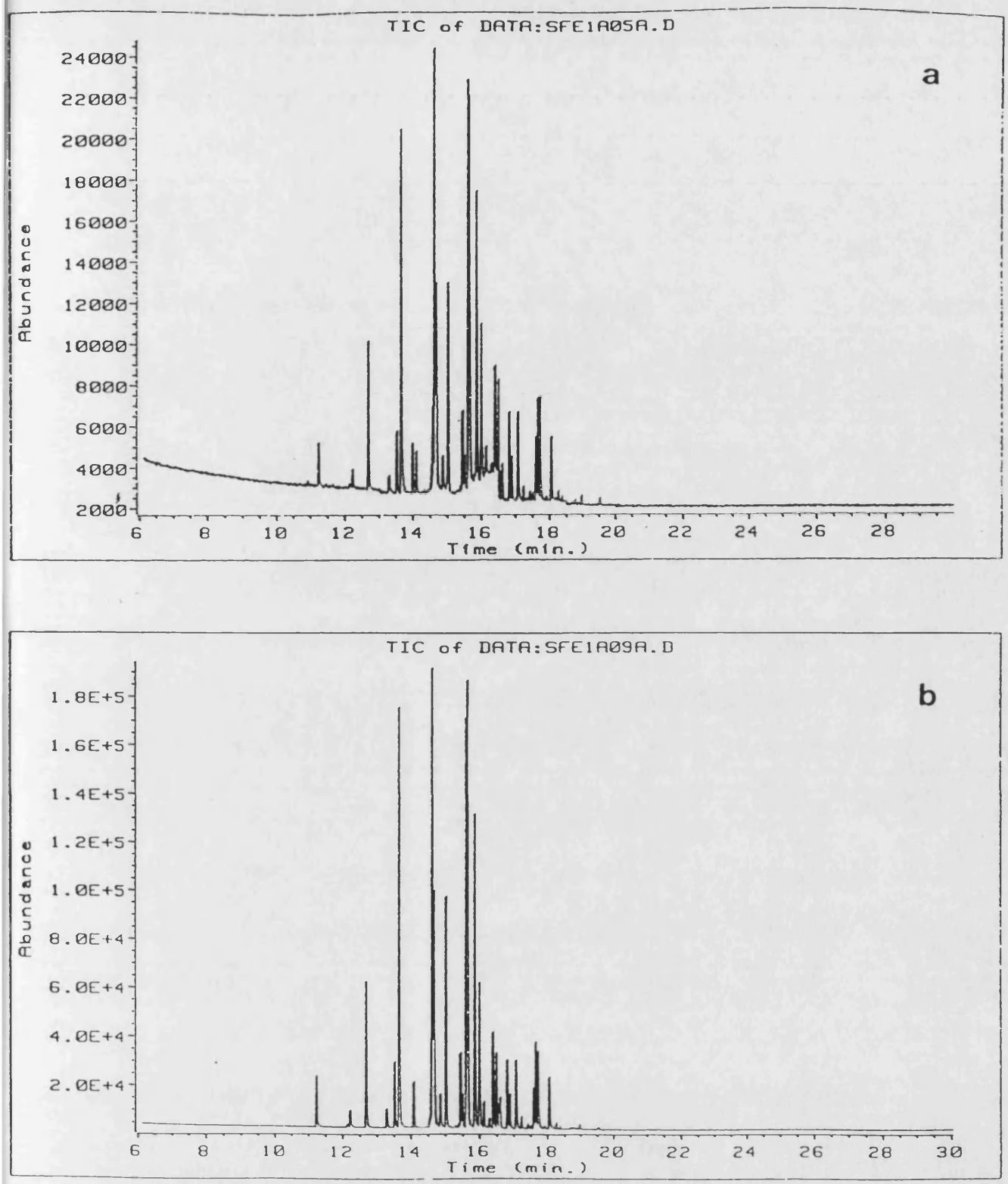


DIAGRAM 7.5: COMPARISON OF GC-MS CHROMATOGRAMS OF ONE OF THE SFE EXTRACTS FROM MILK AND AN AROCLOR 1242 STANDARD

a. GC-MS OF EXTRACT AT 45 ^{o}C:240 kgf/cm^{2}
b. GC-MS OF AROCLOR 1242 STANDARD

This proved that the peak being monitored between 5 and 10 minutes on the UV detector was due to the extraction of the Aroclor 1242 spike. The GC-MS trace also showed no discernable discrimination in the extraction process of PCBs according to their level of chlorination. The GC-MS chromatograms showed that all the different PCB congeners in Aroclor 1242, from monochlorinated to pentachlorinated, were extracted under each set of conditions investigated during the simplex optimisation process. Diagram 7.5 shows a comparison of the chromatogram obtained from the extraction at 240 kgf/cm^2 and 45 ^oC, and a chromatogram of the Aroclor 1242 standard. The two chromatograms are essentially identical, with some very minor differences in the pattern of peaks observed.

In addition, a sample of Aroclor 1260, pentachlorinated to nonachlorinated PCB congeners, was shown to have been extracted from freeze-dried milk and Florisil at 220 kgf/cm^2 and 47 ^oC (point 9). This proved that the full range of PCB homologues, from monochlorinated to nonachlorinated, when spiked onto a matrix of freeze-dried milk and Florisil, could be extracted by SFE in under 15 minutes. This was a very encouraging result, which demonstrated the efficacy of SFE in replacing the far lengthier Soxhlet extraction method for the extraction of PCBs from a cows milk matrix.

7.3.1.2: SFE Of Fat From Cows Milk:

The simplex optimisation of the supercritical fluid extraction of fat from cows milk with CO_2 followed the same procedure as that for the extraction of PCBs reported in Section 7.3.1.1, Diagram 7.4. A UV wavelength of 230 nm was used to monitor the fat, rather than the wavelength of 254 nm used in the optimisation of PCB extraction. Additionally, freeze-dried unskimmed milk was used to form the extraction medium with Florisil. The same amounts of milk and Florisil were used as in the optimisation of PCB extraction, the only difference being the presence of a higher fat content in the milk. The unskimmed milk had a fat content of 4 %, which could easily be monitored by the UV detector at 230 nm.

The simplex optimisation procedure was begun by measuring the peak height response in duplicate at the same initial three sets of extraction conditions, as for the optimisation of PCB extraction (points 1, 2, 3, Diagram 7.4). The peaks obtained for the extraction of fat were not as sharp as previously found for the extraction of PCBs. The optimisation procedure was continued in the same manner as reported for the extraction of PCBs. It was noted that the differences in the peak heights recorded during the optimisation of fat extraction were smaller than for the PCB optimisation. Diagram 7.6 shows two of the UV traces obtained for the extraction of fat from cows milk.

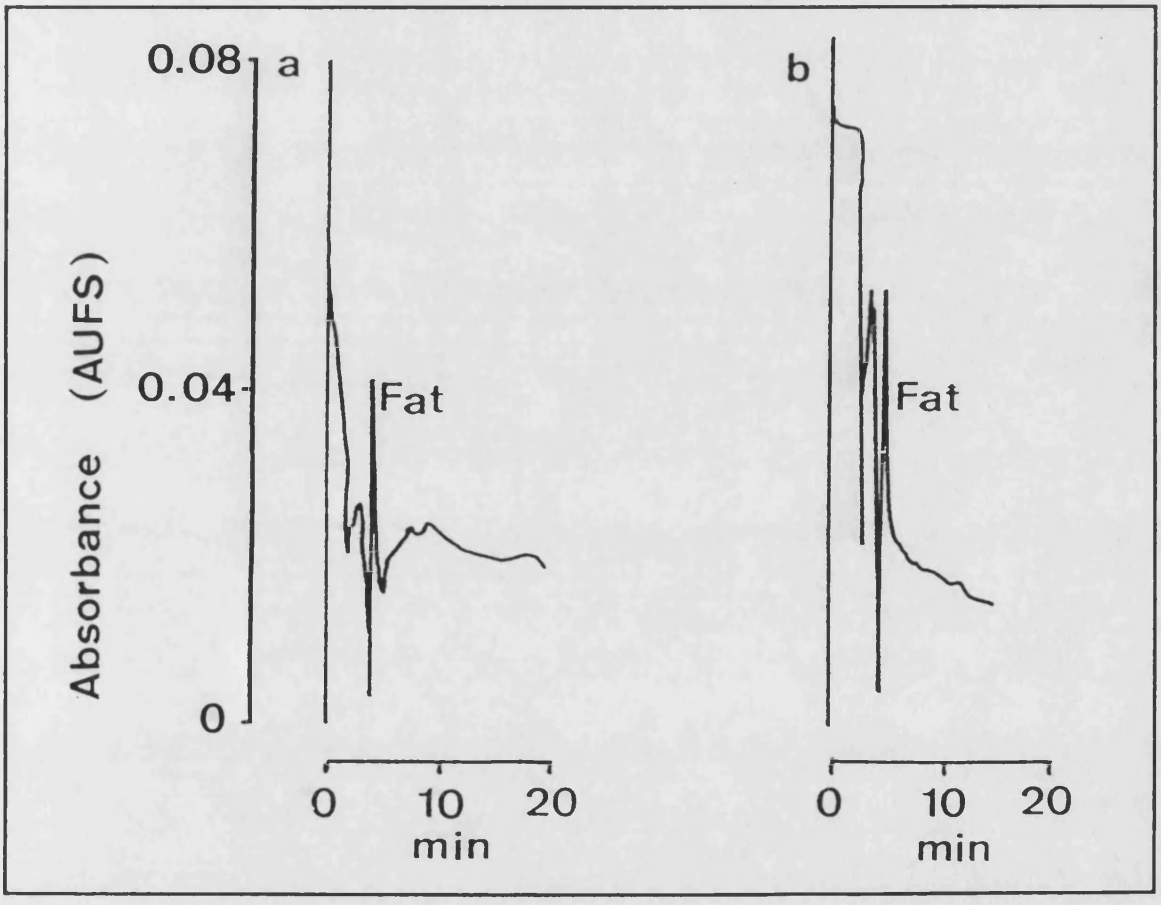


DIAGRAM 7.6: THE SFE OF FAT FROM FREEZE-DRIED MILK

a. 55 ^{o}C:160 kgf/cm^{2} b. 47 ^{o}C:220 kgf/cm^{2}

The optimal conditions for the extraction of fat from cows milk, using SFE with CO_2, were found to be essentially the same as those found for the extraction of PCBs from cows milk by the same method. The optimum extraction conditions again lay within the triangle formed by points 5, 7, and 9 in Diagram 7.4. This illustrated that although SFE using carbon dioxide can readily extract both fat and PCBs from cows milk, the approach reported here was not selective enough to allow the extraction of PCBs from a cows milk matrix without also extracting the fat present in the milk. A second step was, therefore, required to separate the PCBs from the fat.

7.3.2: RESULTS FOR SFC WORK:

7.3.2.1: SFC Of PCBs:

A polymeric PLRP-S column (300 x 7.5 mm i.d.) was the first column to be investigated. This was chosen because this column had been routinely used for a number of years in the laboratory as a semi-preparative HPLC step to isolate PCBs (and pesticides) from milk fat using heptane:2-propanol (35:65 %) as the mobile phase (56). This column was, therefore, selected for the SFC work, as it was known that this column would achieve the required separation under HPLC conditions. The use of the same flowrate for the SFC work as previously used for the HPLC work (2.3 ml/min.) provided a comparison of the two techniques with the PLRP-S column.

A 20 µl sample of 100 ppm Aroclor 1242 in heptane was injected onto the column at 160 kgf/cm^2 and 45 ^oC, with a mobile phase of CO_2:2-propanol (90:10 %) at 2.3 ml/min. Although several peaks were observed on the chart recorder between 4 and 10 minutes, the eluent, collected for 20 minutes, showed no peaks due to PCB congeners when analysed by GC-MS and GC-ECD. An injection of blank heptane gave the same series of peaks between 4 and 10 minutes on the chart recorder. This indicated that the Aroclor 1242 being injected was probably not eluting from the column during the 20 minute collection time. A sample of fat was also found to give the same set of peaks as the injection of blank heptane. Therefore, stronger conditions were employed to try to elute the PCBs from the PLRP-S column. The back pressure was increased to 190 kgf/cm^2, and

then 200 kgf/cm^2, with no change in the result. The mobile phase was changed to 100 % CO_2, and run at 200 kgf/cm^2, but no PCBs were detected by GC-MS or GC-ECD. Under HPLC conditions, 100 % heptane would elute PCBs with the solvent front from this PLRP-S column. This implies that heptane is more non-polar than supercritical CO_2 under the conditions stated. This contrasts with the statement of Mourier *et al.* (183) that the polarity of supercritical CO_2 is close to that of hexane. The conclusion drawn from these results was that the Aroclor 1242 was not eluting from the PLRP-S column under the SFC conditions tried. Therefore, a column expected to show less retention of PCBs was needed.

A 100 x 2.1 mm i.d. RP-8 Spheri-5 column was selected for investigation, as PCBs were expected to elute easily from this short reverse phase column. When a 20 µl injection of 100 ppm Aroclor 1242 in heptane was made at 180 kgf/cm^2 and 50 ^oC, with a mobile phase of 100 % CO_2 at 1 ml/min, a peak was detected at about 5 minutes. GC-MS analysis of the collected eluent proved that this peak was due to the Aroclor 1242. Although this result was encouraging, the Aroclor 1242 was eluting with the solvent front. Therefore, the experimental conditions were varied to try and achieve some retention of the PCBs. The back pressure was reduced to 160 kgf/cm^2, and then 140 kgf/cm^2, and the flowrate was reduced to 0.5 ml/min. However, even with these modifications and a mobile phase of CO_2:2-propanol (40:60 %), it was not possible to increase the retention of the Aroclor 1242 to any degree. Therefore, separation of Aroclor 1242 from fat using this column would not be possible.

These results showed that an HPLC column with more retention for PCBs than the 10 cm RP-8 column, but less retention than the 30 cm PLRP-S column, was needed. In view of previous experience in this laboratory with the polymeric PRP-1 material, a 15 cm x 4.1 mm i.d. column was tested under a range of experimental conditions. After a number of injections at different experimental conditions, a set of peaks were detected between 6 and 20 minutes, when a 20 µl sample of 100 ppm Aroclor 1242 in heptane was injected onto the PRP-1 column, at 160 kgf/cm^2 and 50 ^oC. The mobile phase was CO_2:2-propanol (80:20 %) at a flowrate of 1 ml/min, Diagram 7.7. The eluent was collected as two fractions, labelled (i) and (ii) in Diagram 7.7.

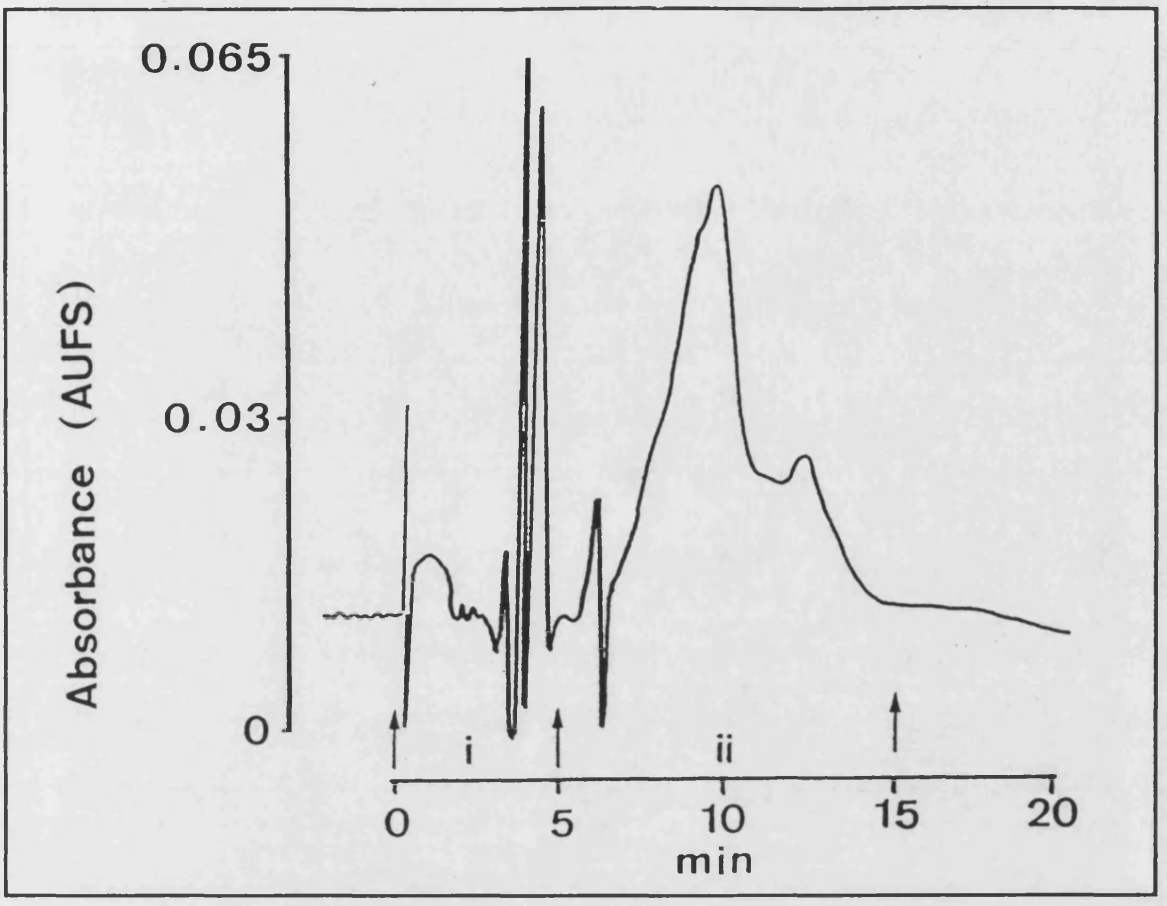


DIAGRAM 7.7: THE SFC OF A 20 µl SPIKE OF 100 ppm AROCLOR 1242 ON A 15 cm PRP-1 COLUMN WITH A MOBILE PHASE OF CO_2:2-PROPANOL (80 %:20 %) AT 50 ^{o}C:160 kgf/cm^2

When the two fractions were analysed by GC-ECD, fraction (i) was found not to contain PCBs, while fraction (ii) showed the Aroclor 1242 pattern.

The back pressure was reduced to 140 kgf/cm^2, and several different mobile phase compositions were used to try and improve the separation of the Aroclor 1242 from the solvent front, and achieve sharper peaks. It was found that a mobile phase of CO_2:2-propanol (80:20 %) gave the most acceptable results.

Fraction collection was used to discover the time range over which the PCBs were eluting. Five fractions were collected from an injection of 20 µl of 100 ppm Aroclor 1242, at 140 kgf/cm^2

and 50 °C, with a mobile phase of CO_2:2-propanol (80:20 %) at a flowrate of 1 ml/min, these are shown in Diagram 7.8.

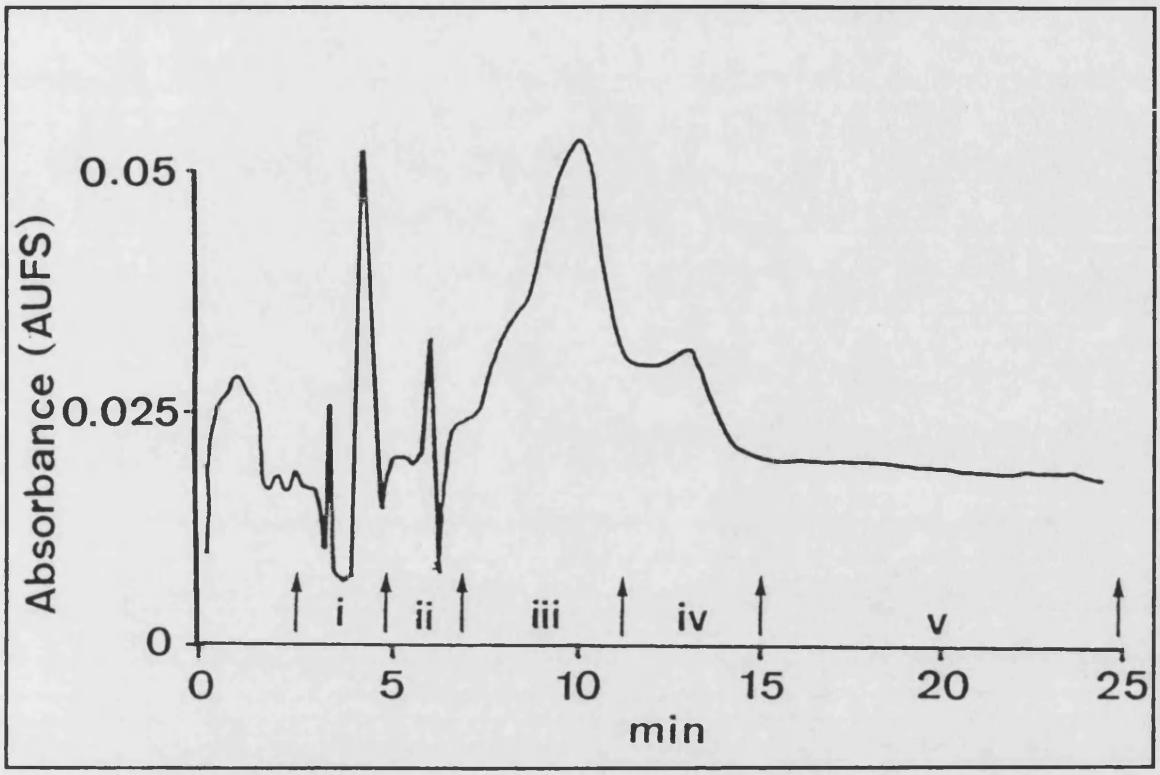


DIAGRAM 7.8: THE SFC OF AROCLOR 1242 - FRACTION COLLECTION WITH A MOBILE PHASE OF CO_2:2-PROPANOL (80 %:20 %) AT 50 °C:140 kgf/cm^2

The five fractions were analysed by both GC-MS and GC-ECD. PCBs were found to be present in fractions (iii) and (iv), while fractions (i), (ii), and (v) showed no evidence of PCBs. Therefore, PCBs were eluting from the 15 cm PRP-1 column, under the specified conditions, between 7 and 15 minutes after injection approximately. This elution behaviour was very similar to that achieved with the PRP-1 column under HPLC conditions. These results indicated that it may prove possible to achieve a separation of fat and PCBs by SFC, using this 15 cm PRP-1 column. The behaviour of fat on this column was subsequently investigated.

7.3.2.2: SFC Of Fat:

Witepsol S55 is a typical lipid material, similar in composition to milk fat (56). This was used to investigate the SFC behaviour of fat. Initially, the level of Witepsol S55 spike needed to give a clearly detectable peak on the chart recorder was determined. A wavelength of 230 nm was used to monitor the fat. A 20 µl spike of 100 mg/ml Witepsol S55 in heptane was found to give a reasonable peak size. The chromatographic conditions used for the fraction collection work with the Aroclor 1242 spike were then adopted. These involved a back pressure of 140 kgf/cm^2 and a temperature of 50 ^{o}C, with a mobile phase of CO_2:2-propanol (80:20 %) at a flowrate of 1 ml/min. The injection of the fat then provided a direct comparison with the PCB results obtained earlier. When the fat spike was injected, a peak was detected at about 4 minutes, the same elution time as a blank injection of heptane.

The identity of the fat peak was confirmed by injecting fat samples of different concentrations. Peaks of different heights were observed at about 4 minutes.

These results showed that the fat eluted before the PCBs from the 15 cm PRP-1 column, under the same chromatographic conditions.

7.3.2.3: SFC Of Fat + PCBs:

In order to confirm the above findings, a combined sample containing fat and PCBs was prepared, by mixing 50 µl of 100 ppm Aroclor 1242 with 50 µl of 100 mg/ml Witepsol S55. A 20 µl sample of this mixture was injected onto the 15 cm PRP-1 column at 140 kgf/cm^2 and 50 ^{o}C, with the mobile phase composition of CO_2:2-propanol (80:20 %) at a flowrate of 1 ml/min. The expected fat and PCB fractions were collected separately. The PCB fraction was analysed by GC-ECD, and the expected Aroclor 1242 pattern was found. The mobile phase composition was then varied to achieve the best possible separation of fat from PCBs.

Two different mobile phase compositions were found to give good SFC results, and were investigated more closely. The two mobile phase compositions were CO_2:2-propanol (80:20 %) and (60:40 %). With each of the mobile phases, the mixture of fat and PCBs showed one large

peak at about 4 minutes, followed by a series of peaks between 6 and 15 minutes. The previous work indicated that the first peak was due to the solvent and fat, and that the later peaks were due to the Aroclor 1242. The "fat" and "PCB" fractions were collected for each mobile phase, Diagrams 7.9 and 7.10.

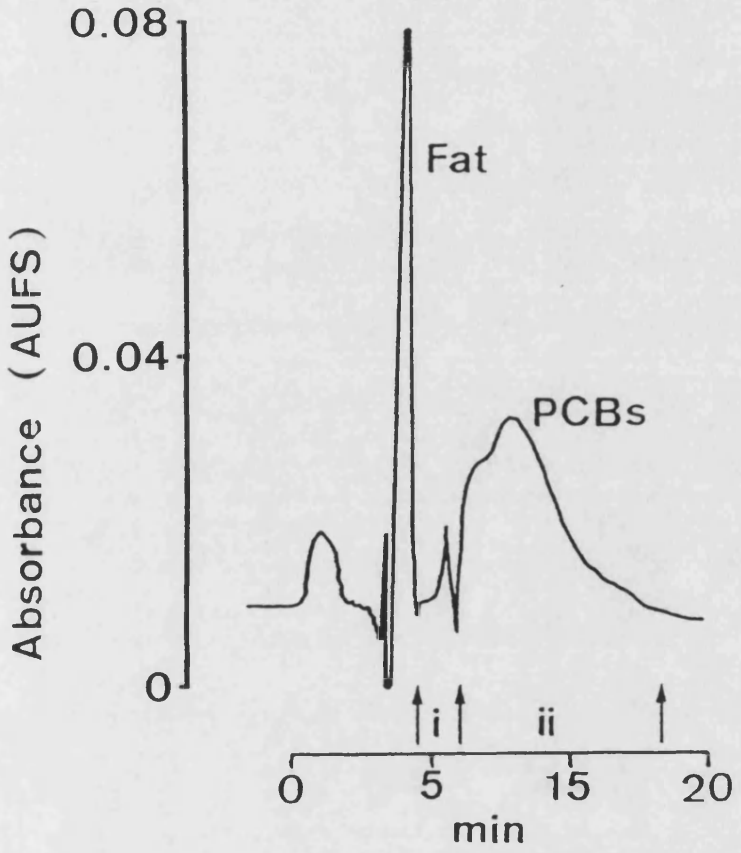


DIAGRAM 7.9: THE SFC OF FAT AND PCBS ON A 15cm PRP-1 COLUMN WITH A MOBILE PHASE OF CO_2:2-PROPANOL (80 %:20 %) AT 50 ^oC:140 kgf/cm^2

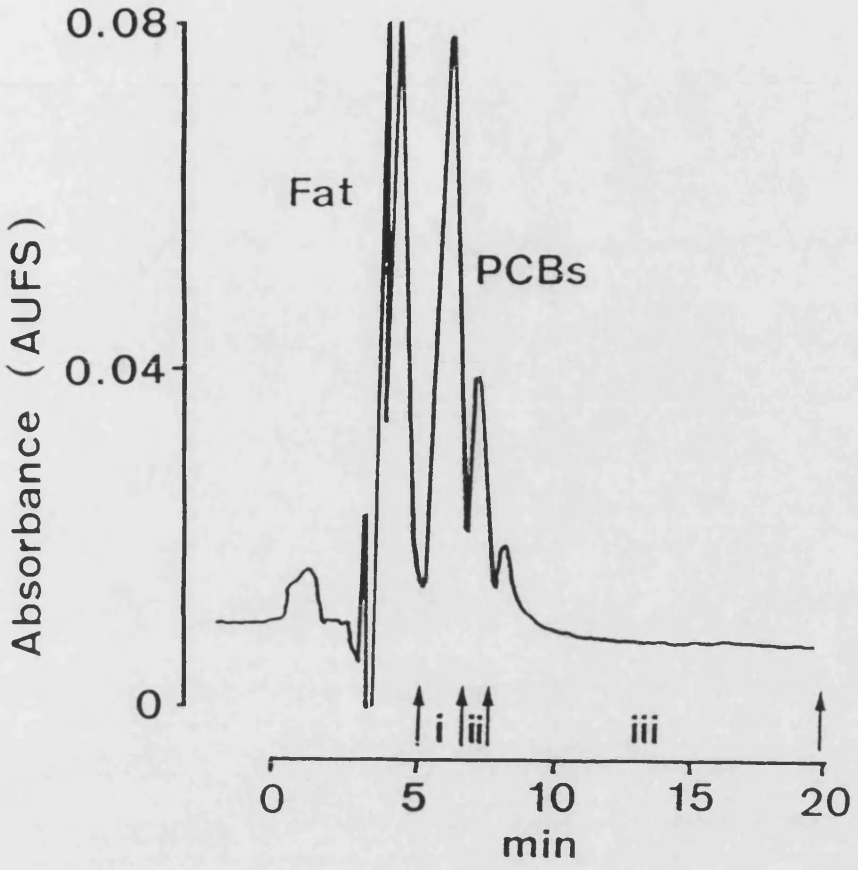


DIAGRAM 7.10: THE SFC OF FAT AND PCBS ON A 15cm PRP-1 COLUMN WITH A MOBILE PHASE OF CO_2:2-PROPANOL (60 %:40 %) AT 50 ^oC:140 kgf/cm^2

The identity of the peak at 4 minutes was confirmed by varying the concentration of fat injected onto the column. Two "PCB" fractions, (i, ii, Diagram 7.9), were collected with the CO_2:2-propanol (80:20 %) mobile phase. Three "PCB" fractions, (i - iii, Diagram 7.10), were collected with the CO_2:2-propanol (60:40 %) mobile phase. All of these fractions were analysed by both GC-MS and GC-ECD. The GC-MS traces obtained are shown in Diagrams 7.11 and 7.12.

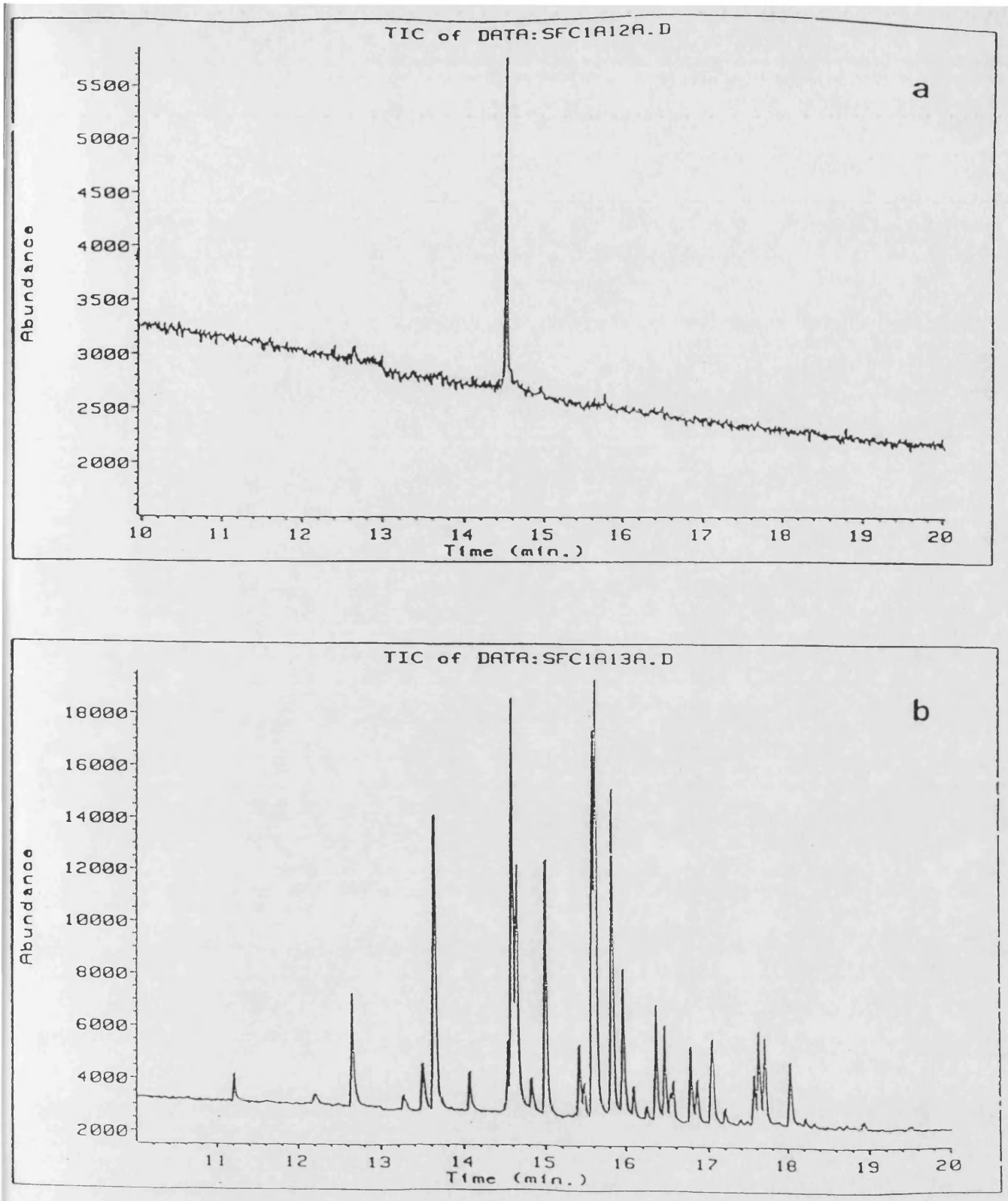
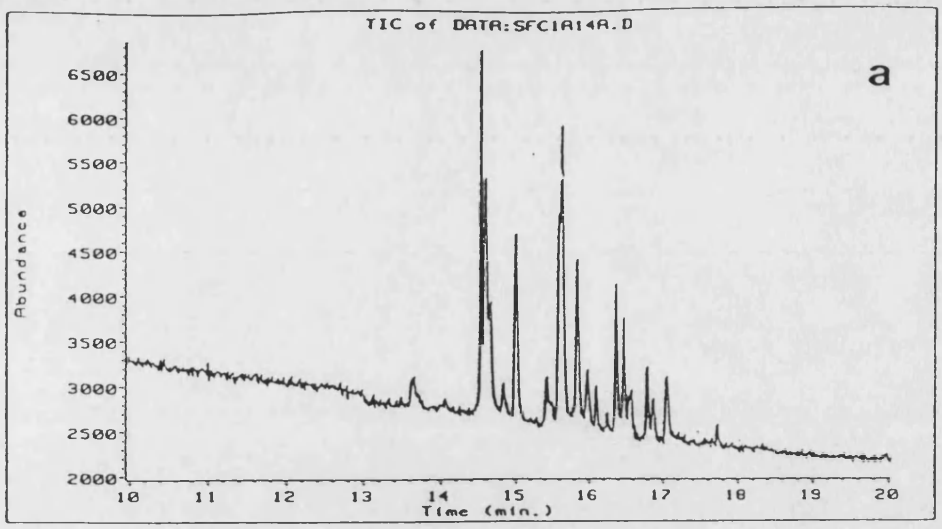
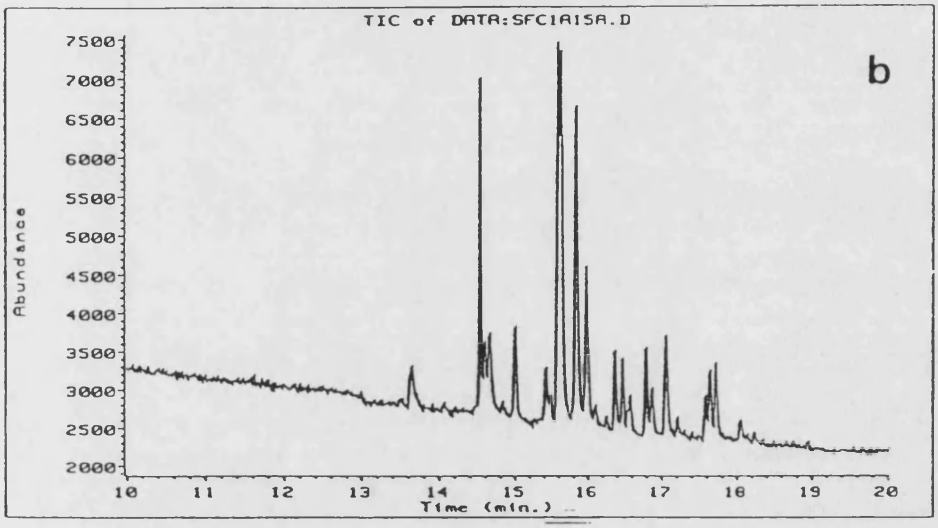


DIAGRAM 7.11: THE GC-MS CHROMATOGRAMS OF THE "PCB" FRACTIONS COLLECTED IN DIAGRAM 7.9

a. "PCB" FRACTION (i) b. "PCB" FRACTION (ii)





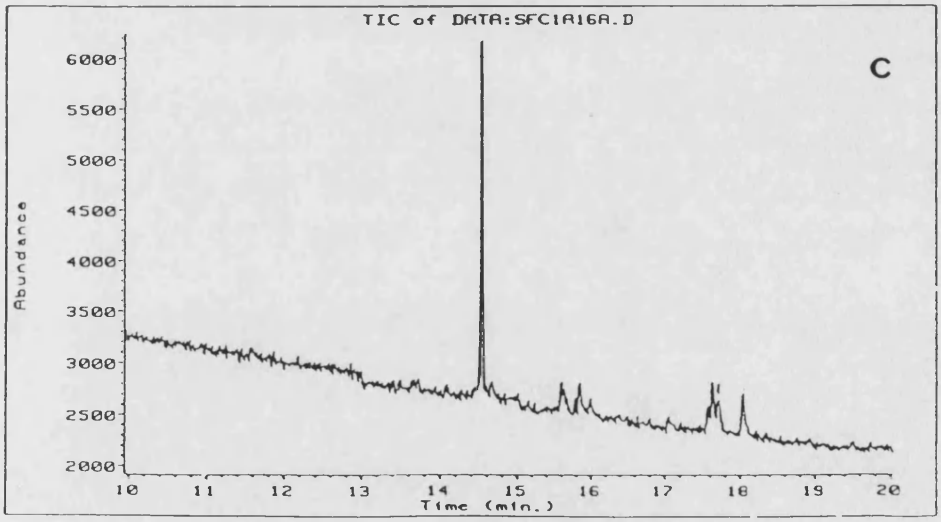


DIAGRAM 7.12: THE GC-MS CHROMATOGRAMS OF THE "PCB" FRACTIONS COLLECTED IN DIAGRAM 7.10

a. "PCB" FRACTION (i) b. "PCB" FRACTION (ii) c. "PCB" FRACTION (iii)

The eluent from the 80:20 % mobile phase showed PCBs to be present only in fraction (ii) (Diagrams 7.9 and 7.11b). In contrast, the eluent from the 60:40 % mobile phase showed PCBs to be present in fractions (i), (ii), and (iii) (Diagrams 7.10 and 7.12a-c).

The separation of the fat from the PCBs was achieved with both mobile phases, with the 80:20 % mobile phase giving the better separation of the fat from the PCBs. This was because the fat peak with the 80:20 % mobile phase was sharper than that with the 60:40 % mobile phase. This meant that there would be less likelihood of the presence of traces of fat in the collected PCB fraction with the 80:20 % mobile phase, than with the 60:40 % mobile phase. This was extremely important because, from previous experience, the presence of trace levels of fat in PCB extracts has a deleterious effect on the column performance of a GC-MS. This leads to a rapid increase in baseline noise, resulting in a reduction in MS sensitivity.

The 60:40 % mobile phase showed some chromatographic resolution between PCBs, probably based upon differences in levels of biphenyl chlorination. Aroclor 1242 is made up of a large number of PCB congeners, from monochlorinated to pentachlorinated biphenyls. Diagram 7.12a shows that mostly monochlorinated to trichlorinated PCB congeners eluted between 5 and 7 minutes, directly after the elution of the fat, with the higher chlorinated congeners eluting after longer than 7 minutes (Diagrams 7.12b and 7.12c). These early eluting congeners are the ones which may be contaminated with traces of fat. In contrast, the 80:20 % mobile phase shows that no PCBs are eluting for the first couple of minutes after the elution of the fat (Diagram 7.11a). All of the PCB congeners, from monochlorinated to pentachlorinated, eluted after longer than 6 minutes (Diagram 7.11b). The peak at 14.5 minutes in Diagrams 7.11 and 7.12 was not a PCB peak, but probably a silyl fragment from the stationary phase of the capillary GC column.

These experimental results proved that it was possible to separate fat from PCBs by supercritical fluid chromatography on a 15 cm PRP-1 column. SFC should be a preferable separation technique to HPLC for PCBs in fatty food samples. This is because SFC does not require the use of large amounts of hazardous, toxic organic solvents, or the further concentration

of samples prior to GC analysis. Both of these requirements are inherent drawbacks of HPLC methodology.

The work reported above was done on a qualitative basis, as an initial investigation into the possible use of supercritical fluids for the extraction and separation of PCBs from fatty food matrices. The work reported above has shown the feasibility and ease of use of supercritical fluids for this task. A full quantitative study of both the SFE and SFC of PCBs is now required.

7.3.3: RESULTS FOR COUPLED SFE-SFC WORK:

Some different columns were tested for their effectiveness as trapping columns. A 100 x 2.1 mm i.d. Brownlee Labs. RP-8 Spheri-5 column (Anachem, Luton, G.B.), used in the initial SFC work, was tested. The first thing to investigate was whether this column would trap PCBs extracted by the SFE system. Therefore the system of Rheodyne valves was set up for SFE, and the RP-8 Spheri-5 column was attached to the outlet from the back pressure regulator. The trapping column was immersed in ice. 50 µl of 100 ppm Aroclor 1242 was spiked onto Florisil and extracted at 210 kgf/cm^2 and 47 °C, with 100 % CO_2 at 3 ml/min. A piece of tubing attached to the far side of the trapping column was immersed in solvent in an ice-cooled test tube. The extraction was carried out for 30 minutes, and a peak on the chart recorder for the extraction of the Aroclor 1242 was seen. The solvent in the test tube from the far side of the trapping column was run on the GC-MS. This was to test whether the trapping column was indeed retaining the extracted PCBs, or if the PCBs were escaping from the end of the trapping column. It was found that the column was not retaining the extracted PCBs. Therefore, a column expected to retain PCBs more strongly than the RP-8 Spheri-5 column was needed.

A 10 cm x 4.6 mm i.d. Lichrosorb RP-8 column (Fisons Sci Equip., Loughborough, G.B.) was then tried as a trapping column. This column was larger in volume than the Brownlee RP-8 Spheri-5 column previously tested, and, therefore, would be expected to retain the extracted PCBs more strongly. When the same extraction of PCBs as for the earlier column was performed, no evidence of the PCBs escaping from the far end of the column was found. A peak was observed on

the chart recorder for the extraction of the PCBs, and it was, therefore, concluded that the Lichrosorb RP-8 column was indeed trapping the extracted PCBs. The trapping column was then immersed in the water bath, and the Rheodyne valves switched, to allow the CO_2 to pass through the trapping column, and then through the SFC column. The SFC experiment was carried out at 140 kgf/cm^2 and 50 ^oC, with a mobile phase of CO_2:2-propanol (80:20 %) at 1 ml/min. Three fractions were collected from the SFC run. Fractions were collected between 0 and 20 minutes, 20 and 40 minutes, and 40 and 60 minutes. All three fractions were run on the GC-MS, and PCBs were only found in the fraction collected between 40 and 60 minutes. This implied that the Lichrosorb RP-8 column was probably retaining the PCBs too strongly to be entirely suitable as a trapping column. A fat sample extracted by SFE was also shown to be successfully trapped by the Lichrosorb RP-8 column.

It was proposed that a short, fat column with either RP-8 or RP-18 packing material might be better suited as a trapping column for the coupled SFE-SFC system.

7.4: DISCUSSION:

The work presented shows the effectiveness of supercritical fluids for the extraction of PCBs and fat from cows milk, and for the separation of the extracted PCBs and fat. The current methods for the extraction of PCBs from cows milk in the literature usually employ a Soxhlet extraction step, followed by a chromatography step to separate the PCBs from fat and other co-extractants.

This traditional approach has three main disadvantages when compared to methods employing supercritical fluids:

(i) A large volume of toxic organic solvents is needed.

(ii) The time taken for the extraction is lengthy.

(iii) Additional pre-concentration steps are needed prior to the final analysis, increasing the possibility of sample loss or contamination.

The proven speed, efficiency, and ease of use of supercritical fluids for extraction and chromatographic separation, coupled with the increased availability of supercritical fluid equipment, points to the increased utilisation of supercritical fluids on a routine basis for the analysis of trace organics in milk and, probably, a wide range of other matrices.

CHAPTER 8: COMPARISON OF DIFFERENT QUANTITATION METHODS FOR CAPILLARY COLUMN GC-MS AND GC-ECD ANALYSES OF PCBS

A total of six samples were analysed by both capillary column GC-MS and capillary column GC-ECD using a number of different quantitation methods. The six samples that were analysed consisted of three human breast milk samples and three cows milk samples. The results obtained by the different quantitation methods were statistically analysed.

8.1: QUANTITATION METHODS:

8.1.1: GC-MS QUANTITATION METHODS:

All of the samples were analysed by capillary column GC-MS. An HP5890 gas chromatograph with a 5970 MSD mass spectrometer and a 7673A automatic sampler was used (Hewlett-Packard, Bracknell, G.B.). A 50 m x 0.22 mm i.d. BPX-5 column (SGE (UK) Ltd., Milton Keynes, G.B.) with a film thickness of 0.25 μm was used for the analyses. Hydrogen was the GC carrier gas, with a volumetric flow rate of 1 ml/min. Manual, cold on-column injection was used, with 2 μl of each sample injected by a syringe with a fused silica needle. A temperature programme was used for the analyses of the six samples. This consisted of an initial temperature of 75 ^{o}C held for 2 minutes, followed by a temperature ramp of 30 ^{o}C/min to 150 ^{o}C. This was immediately followed by a temperature ramp of 2.5 ^{o}C/min to 270 ^{o}C. The final temperature of 270 ^{o}C was held for 20 minutes.

The SIM programme that was used meant that at any given time during a GC analysis the mass spectrometer was searching for 4 specific mass values. The 4 mass values represented two masses for each of two levels of chlorination.

8.1.1.1: ICES Method:

Three different quantitation methods were used for the capillary column GC-MS results. The first of these was an adaptation of the ICES method outlined earlier in Section 2.3.2.1. This used a standard solution, called PCB Mix No. 3, containing the 7 individual PCB congeners No. 28, 52, 101, 118, 138, 153, and 180 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), each at a concentration of 10 ng/μl, in iso-octane.

A set of six calibration solutions were made with concentrations of the 7 PCB congeners of 100 ppb, 70 ppb, 50 ppb, 35 ppb, 25 ppb, and 10 ppb. However, unlike the method detailed in Section 2.3.2.1, this set of calibration solutions contained 4, 4'-dibromobiphenyl as the internal standard at a concentration of 1 ppm. The quantitation of the samples was made relative to the 4, 4'-dibromobiphenyl. Each of the standards was run on the capillary GC-MS, using a SIM programme.

Identification of the PCB congeners in the six samples was done by comparison of the experimental retention times of the peaks in the samples with those of the standards. A sample peak was said to be due to one of the 7 congeners of interest if the retention time of the sample peak equalled the retention time of the standard peak to within +/- 0.1 min.

Once the sample peaks of interest had been identified, and their experimental peak areas recorded, the concentrations of the 7 individual PCB congeners of interest in each sample were calculated using the "ICES" method, as detailed below:

The method involved calculating the relative response factors (RRF) for each of the 7 target PCB congeners using equation (1):

$$RRF = \frac{A_2 \times M_1}{A_1 \times M_2} \qquad - (1)$$

where, A_2 = peak area of target congener

M_1 = mass of 4, 4'-dibromobiphenyl

A_1 = peak area of 4, 4'-dibromobiphenyl

M_2 = mass of target congener

The RRFs were calculated for each of the 7 individual PCB congeners in each of the PCB Mix No.3 standard solutions, and the mean RRF values for each congener were used for the quantitation work. The concentrations of each PCB congener in a sample were calculated using equation (2):

$$C_i = \frac{C_S \times A_i \times RRF_S}{A_S \times RRF_i} \qquad - (2)$$

where, C_i = concentration of congener i

C_S = concentration of 4, 4'-dibromobiphenyl

A_i = peak area of congener i

A_S = peak area of 4, 4'-dibromobiphenyl

RRF_i = RRF of congener i

RRF_S = RRF of 4, 4'-dibromobiphenyl = 1

The peak areas A_i and A_S were measured and reported by the GC-MS, the concentration of 4, 4'-dibromobiphenyl, C_S, was known, and the RRFs of each congener, RRF_i, were known. Therefore, the concentrations of each of the 7 congeners, C_i, could be calculated using equation (2).

The concentrations of each of the 7 individual PCB congeners in the six samples were calculated. These results were then divided by the weight of each sample that had been extracted. This gave a concentration for each of the 7 congeners in each sample. A Total PCB concentration for each sample was then calculated by adding together the concentrations for the 7 individual congeners, and multiplying the answer by a factor of four, as recommended by the ICES Method, i.e. Total PCBs = (PCB No. 28 + 52 + 101 + 118 + 138 + 153 + 180) x 4.

8.1.1.2: "PCB Profile" Method:

The second quantitation method used was the "PCB Profile" method outlined in Section 2.1.2. The quantitation of the PCBs present was achieved by using an external calibration standard called RPCBR-1 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), which contained two mono-substituted PCB congeners and one congener for each of the other nine chlorination levels. The standard solution supplied by Greyhound contained eleven individual PCB congeners, each at a concentration of 1000 μg/ml. The eleven PCBs present were No.s 1, 3, 7, 30, 50, 97, 143, 183, 202, 207, and 209. Each PCB congener selected had a GC response factor which was representative of the response factors of the other PCB congeners of that chlorination level.

A set of solutions of this RPCBR-1 standard at different concentrations were made up and run on the GC-MS using the SIM programme specially developed for PCBs. Six calibration solutions were prepared, containing the PCB congeners at concentrations of 1 μg/ml, 700 ng/ml, 500 ng/ml, 400 ng/ml, 250 ng/ml, and 100 ng/ml, respectively. All of the standards had 4,4'-dibromobiphenyl present as an internal standard at a concentration of 1 μg/ml (or 1 ppm), exactly as in the six samples. The six standard solutions were run on the GC-MS.

Calibration curves for each of the ten chlorination levels were constructed, with the peak area of the PCB congener of interest divided by the peak area of the internal standard (4,4'-dibromobiphenyl) and plotted against the known concentrations of the RPCBR-1 solutions. Each

calibration curve was plotted on a computer using a statistical package called INSTAT. This statistical package calculated the calibration curve, the line of regression of y on x, by the least squares method, and reported the correlation coefficient, r. The computer also reported the slope (m) and intercept (c) of the calibration curve, from the general equation for a straight line, $y = mx + c$.

These ten calibration curves were then used to calculate the amounts of PCBs in the samples by chlorination level (homologue). The use of an internal standard allowed for any variation in the actual volume of sample injected by the GC autosampler.

The SIM programme used for these analyses ensured that each peak recorded by the GC-MS had to pass a test before being accepted as a PCB peak. At each level of chlorination, peak areas at two different masses were recorded. The ratio of the two peak areas had to fall within pre-set limits (Tables 2.1a & b), which were different for each level of chlorination, before any peak was accepted as a PCB peak. If the experimental peak area ratio was greater than +/-20 % of the theoretical value, the peak was rejected from the quantitation procedure. Only the peak areas from one mass value, the primary ion, were used to quantify the PCBs at each level of chlorination.

The results calculated for each of the six samples were divided by the weight of sample extracted to give the final concentration. The results were reported as a Total PCB concentration, obtained by summing all of the individual results, as well as by chlorination level.

8.1.1.3: Use Of A 51 Congener PCB Standard:

The 51 individual PCB congeners were supplied in four solutions labelled CLB-1-A, CLB-1-B, CLB-1-C, and CLB-1-D by the Marine Analytical Chemistry Standards Program at the National Research Council of Canada (Halifax, Nova Scotia, Canada). Each of the four solutions contained either 14 or 15 individual PCB congeners, and 1 ml of each solution was supplied in a sealed glass ampule. The concentrations of all of the PCB congeners in each solution were supplied with the solutions, and are shown in Table 8.1.

CLB-1 SOLUTION A		CLB-1 SOLUTION B		CLB-1 SOLUTION C		CLB-1 SOLUTION D	
PCB No.	CONC (µg/ml)	PCB No.	CONC (µg/ml)	PCB No.	CONC (µg/ml)	PCB No.	CONC (µg/ml)
18	11.8	15	91.9	15	138.1	15	76.7
31	6.6	52	15.2	114	6.3	101	8.9
40	4.9	60	3.9	129	8.3	118	3.9
44	5.9	103	10.8	137	7.4	138	4.2
49	7.6	105	4	153	7.3	141	2.8
54	16.6	128	4.9	171	5.2	151	5
77	5.5	143	5.7	183	6.6	153	3.3
86	2.9	154	6.2	185	3.5	170	3
87	3.8	173	2.3	189	4.7	180	2.8
121	3.1	182	3.8	191	5	187	3.2
153	2.1	202	3.6	199	4.8	194	2.4
156	1.5	205	3.2	201	7	195	2.6
159	1.2	207	3.8	203	5.1	196	3.3
209	1.7	208	2.4	206	6.7	199	3.6
		209	2.8	209	5.1	209	2.7

TABLE 8.1: CONCENTRATIONS OF PCB CONGENERS IN CLB-1

From Marine Analytical Chemistry Standards Program, Nova Scotia, Canada.

A 900 µl aliquot of each solution was measured out into a separate vial using a graduated syringe. 100 µl of a 10 ppm solution of 4, 4'-dibromobiphenyl, as an internal standard for quantitation purposes, was added to each vial. The four solutions were then analysed by capillary column GC-MS. The peak areas of each PCB congener present were divided by the internal standard. These peak area ratios were then used to quantify the levels of the 51 individual PCB congeners in the six milk samples. This was possible because the six milk samples contained the same concentration of 4, 4'-dibromobiphenyl as the four standard solutions. The retention times of the standards were used to identify the individual congeners present in the milk samples.

Each milk sample was run on the GC-MS, and the retention times of all the peaks found were compared with those of the standards. A sample peak was identified as one of the 51 congeners in the standards if the experimental retention time matched that of a standard congener +/- 0.1 minutes. The measured peak area was then divided by the peak area of the internal standard, and converted to a concentration using the known peak area ratio of a specified concentration in the standard.

There were several pairs of congeners in the 51 congener set that were not fully resolved using the chromatographic conditions given in Section 8.1.1. These are listed in the results section. No octachlorobiphenyls or nonachlorobiphenyls were found in the six milk samples. PCB No. 15 was not quantified in the six milk samples. This was because it was present in the standards at such high concentrations that a consistent peak area ratio result could not be achieved, due to overloading of the chromatographic column.

8.1.2: GC-ECD QUANTITATION METHOD:

All of the milk samples were also analysed by capillary column GC-ECD. An 8320B capillary column GC-ECD (Perkin-Elmer, Beaconsfield, G.B.) with a ^{63}Ni electron capture detector was used. A 30 m x 0.25 mm i.d. DB-5 column (Jones Chromatogr., Hengoed, G.B.) with a film thickness of 0.25 μm was used for the analyses. The splitless injector temperature was set at 250 ^{o}C, and the detector temperature at 290 ^{o}C. Hydrogen was used as the carrier gas, at a pressure of 6 p.s.i., with nitrogen as the make-up gas. A temperature programme was developed for the analyses. This consisted of an initial temperature of 50 ^{o}C for 1 minute, followed by temperature ramps of 30 ^{o}C/min to 150 ^{o}C and then, 2.5 ^{o}C/min to 290 ^{o}C. The final temperature of 290 ^{o}C was held for 10 minutes. All of the chromatograms were plotted out on a GP-100 graphics plotter (Perkin-Elmer, Beaconsfield, G.B.), and the peak heights were measured manually.

The milk samples were quantified using exactly the same method, the "ICES" method, that was described in Section 8.1.1.1. The same set of six solutions of the 7 congener standard, PCB

Mix No. 3 (British Greyhound Chromatography & Allied Chemicals, Birkenhead, G.B.), were used. The six calibration solutions contained concentrations of the 7 PCB congeners of 100 ppb, 70 ppb, 50 ppb, 35 ppb, 25 ppb, and 10 ppb, as well as 4, 4'-dibromobiphenyl at 1 ppm, PCB No.30 at 0.515 ppm and PCB No.209 at 0.44 ppm. The individual peak heights of each congener were divided by the peak height of the 4, 4'-dibromobiphenyl, and the quantitation of the samples was done relative to the 4, 4'-dibromobiphenyl.

Identification of the PCB congeners in the six samples was done by comparison of the experimental retention times of the peaks in the samples with those of the standards. As previously, a sample peak was said to be due to one of the 7 congeners of interest if the retention time of the sample peak equalled the retention time of the standard peak to within +/- 0.1 min.

Once the sample peaks of interest had been identified, and their experimental peak heights measured, the concentrations of the 7 individual PCB congeners of interest in each sample were calculated using the "ICES" method, as detailed below:

The method involved calculating the relative response factors (RRF) for each of the 7 target PCB congeners using equation (3):

$$RRF = \frac{H_2 \times M_1}{H_1 \times M_2} \qquad -(3)$$

where, H_2 = peak height of target congener

M_1 = mass of 4, 4'-dibromobiphenyl

H_1 = peak height of 4, 4'-dibromobiphenyl

M_2 = mass of target congener

The RRFs were calculated for each of the 7 individual PCB congeners in each of the PCB Mix No.3 standard solutions, and the mean RRF values for each congener were used for the quantitation work. The concentrations of each PCB congener in a sample were calculated using equation (4):

$$C_i = \frac{C_S \times H_i \times RRF_S}{H_S \times RRF_i} \quad \text{- (4)}$$

where, C_i = concentration of congener i

C_S = concentration of 4, 4'-dibromobiphenyl

H_i = peak height of congener i

H_S = peak height of 4, 4'-dibromobiphenyl

RRF_i = RRF of congener i

RRF_S = RRF of 4, 4'-dibromobiphenyl = 1

The peak heights H_i and H_S were measured, the concentration of 4, 4'-dibromobiphenyl, C_S, was known, and the RRFs of each congener, RRF_i, were known. Therefore, the concentrations of each of the 7 congeners, C_i, could be calculated using equation (4).

The concentrations of each of the 7 individual PCB congeners in the six samples were calculated. These results were then divided by the weight of each sample that had been extracted. This gave a concentration for each of the 7 congeners in each sample. A Total PCB concentration for each sample was then calculated by adding together the concentrations for the 7 individual congeners, and multiplying the answer by a factor of four, as previously in Section 8.1.1.1, i.e. Total PCBs = (PCB No. 28 + 52 + 101 + 118 + 138 + 153 + 180) x 4.

8.2: RESULTS:

8.2.1: GC-MS RESULTS:

8.2.1.1: GC-MS Results Using ICES Method:

The results obtained for the six milk samples using the ICES Method (Section 8.1.1.1) are shown in Table 8.2. The results for the 7 individual PCB congeners of the ICES Method are represented by the IUPAC number of each congener, e.g. PCB No. 28 denotes the concentration in mg/kg Whole Milk of that congener. The Total PCB results were calculated by adding together the individual congener results of a sample, and multiplying the sum by a factor of four.

PCB No.	Concentration of PCBs in mg/kg Whole Milk					
	Breast Milk Samples			Cows Milk Samples		
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 1	Sample No. 2	Sample No. 3
28	0.0070	0.0012	0.0015	0.0018	0.0044	0.0021
52	0.0040	0.0004	0.0004	0.0013	0.0010	0.0010
101	0.0007	0.0007	0.0002	ND	ND	ND
118	0.0005	0.0007	0.0002	ND	ND	ND
138	0.0003	0.0019	0.0003	0.0005	0.0005	0.0003
153	0.0003	0.0033	0.0004	0.0005	0.0005	0.0004
180	ND	0.0015	0.0002	ND	ND	ND
Total PCBs (ICES)	0.0512	0.0388	0.0128	0.0164	0.0256	0.0152

ND = not detected

TABLE 8.2: RESULTS OBTAINED FROM GC-MS ANALYSIS USING THE ICES METHOD

8.2.1.2: GC-MS Results Using PCB Profile Method:

The results obtained for the six milk samples using the PCB Profile Method (Section 8.1.1.2) are shown in Table 8.3. The results for the PCB Profile Method are represented by chlorination level, e.g. 2Cl denotes the total concentration in mg/kg Whole Milk of dichlorobiphenyls. The Total PCB results were calculated by adding together the results by chlorination level of each sample.

Chlorination Level	Concentration of PCBs in mg/kg Whole Milk					
	Breast Milk Samples			Cows Milk Samples		
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 1	Sample No. 2	Sample No. 3
2Cl	0.0081	0.0051	0.0055	0.0063	0.0082	ND
3Cl	0.0120	0.0036	0.0041	0.0036	0.0089	0.0029
4Cl	0.0124	0.0048	0.0045	0.0047	0.0079	0.0070
5Cl	0.0058	0.0047	0.0047	ND	ND	ND
6Cl	0.0027	0.0067	0.0027	0.0028	0.0032	0.0028
7Cl	ND	0.0049	0.0028	ND	ND	ND
Total PCBs (Profile)	0.0410	0.0298	0.0242	0.0174	0.0282	0.0127

ND = not detected

TABLE 8.3: RESULTS OBTAINED FROM GC-MS ANALYSIS USING THE PCB PROFILE METHOD

8.2.1.3: GC-MS Results Using 51 Congener Method:

The results obtained for the six milk samples using the 51 Congener Method (Section 8.1.1.3) are shown in Table 8.4. No octachlorinated or nonachlorinated PCBs were found in any of the six milk samples. A Total PCB concentration for each sample was calculated by adding together all of the individual congener results for that sample.

Chlorination Level	PCB No.	Concentration of PCBs in mg/kg Whole Milk					
		Breast Milk Samples			Cows Milk Samples		
		Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 1	Sample No. 2	Sample No. 3
3Cl	18	0.0177	0.0037	0.0041	0.0151	0.0169	0.0103
	31	0.0221	0.0037	0.0048	0.0056	0.0138	0.0068
4Cl	52	0.0128	0.0012	0.0012	0.0043	0.0033	0.0031
	49	ND	0.0011	0.0010	ND	0.0041	0.0033
	44	0.0075	0.0012	0.0013	ND	0.0057	0.0042
	40	ND	ND	0.0014	0.0004	ND	0.0011
	60	0.0040	0.0006	0.0004	0.0057	0.0014	0.0032
5Cl	121	0.0011	ND	ND	ND	ND	ND
	101	0.0007	0.0007	0.0002	ND	ND	ND
	86	0.0008	ND	ND	ND	ND	ND
	87	0.0011	0.0011	ND	ND	ND	ND
	118	0.0006	0.0007	0.0002	ND	ND	ND
	114	ND	0.0002	ND	ND	ND	ND
6Cl	154	ND	0.0002	ND	ND	ND	ND
	151	ND	0.0001	ND	ND	ND	ND
	143	0.0001	0.0002	ND	ND	ND	ND
	153	0.0009	0.0089	0.0004	0.0012	0.0014	0.0011
	141	0.0001	ND	ND	ND	ND	ND
	137	ND	0.0002	ND	0.0001	ND	ND
	138	0.0003	0.0020	0.0003	0.0005	0.0005	0.0004
7Cl	187	ND	0.0005	0.0001	ND	ND	ND
	183	ND	0.0003	0.0001	ND	ND	ND
	180	ND	0.0017	0.0002	ND	ND	ND
	170	ND	0.0008	0.0001	ND	ND	ND
Total PCBs (51 Cong.)	-	0.0698	0.0291	0.0158	0.0329	0.0471	0.0335

ND = not detected

N.B. PCBs No. 31 & 28 (ICES) elute very closely together
PCBs No. 49 & 52 elute very closely together
PCBs No. 187 & 182 elute very closely together
PCB No. 103 & I.S. elute very closely together

TABLE 8.4: RESULTS OBTAINED FROM GC-MS ANALYSIS USING THE 51 CONGENER METHOD

8.2.2: GC-ECD RESULTS:

The results for the six milk samples by GC-ECD using the ICES Method are shown in Table 8.5. The Total PCB concentrations from the GC-ECD results were calculated by adding together the seven individual congener concentrations, and multiplying the sum by a factor of four.

PCB No.	Concentration of PCBs in mg/kg Whole Milk					
	Breast Milk Samples			Cows Milk Samples		
	Sample No. 1	Sample No. 2	Sample No. 3	Sample No. 1	Sample No. 2	Sample No. 3
28	0.0052	0.0046	0.0055	0.0081	0.0193	0.0046
52	0.0021	0.0011	0.0015	0.0029	0.0053	0.0024
101	0.0004	0.0003	0.0004	ND	0.0020	ND
118	0.0008	0.0003	0.0008	0.0005	0.0007	0.0017
138	0.0003	0.0009	0.0012	0.0005	0.0007	0.0020
153	0.0005	0.0012	0.0008	0.0025	0.0008	0.0003
180	0.0024	0.0016	0.0131	0.0092	0.0063	0.0038
Total PCBs (GC-ECD)	0.0468	0.0400	0.0932	0.0948	0.1404	0.0592

ND = not detected

TABLE 8.5: CONCENTRATIONS OF 7 INDIVIDUAL PCB CONGENERS BY ICES METHOD USING GC-ECD

8.3: DISCUSSION:

The Total PCB results obtained by the different chromatographic and quantitation methods were compared by displaying as histograms. 6 of the 7 congeners analysed by the ICES Method were also analysed by the 51 Congener Method, and the individual congener results were compared. The results by the 51 Congener Method were also processed by chlorination level and compared with the PCB Profile Method results. The results of the 7 individual congeners used in the ICES Method were compared by GC-MS and GC-ECD.

In addition, the Total PCB results obtained by the different techniques were tested statistically to determine whether the various methods gave Total PCB results that were significantly different. The possible interaction of different techniques and sample types was also investigated. Analysis of variance was used for this work.

8.3.1: COMPARISON OF ICES METHOD AND PCB PROFILE METHOD USING GC-MS:

The Total PCB results obtained by the ICES Method and the PCB Profile Method were shown to be fairly similar when compared visually, as can be seen in Diagram 8.1. Only Breast Milk Sample No. 3 shows a wide difference between the Total PCB results obtained by the two quantitation methods. All the rest of the samples show close agreement between the two Total PCB results.

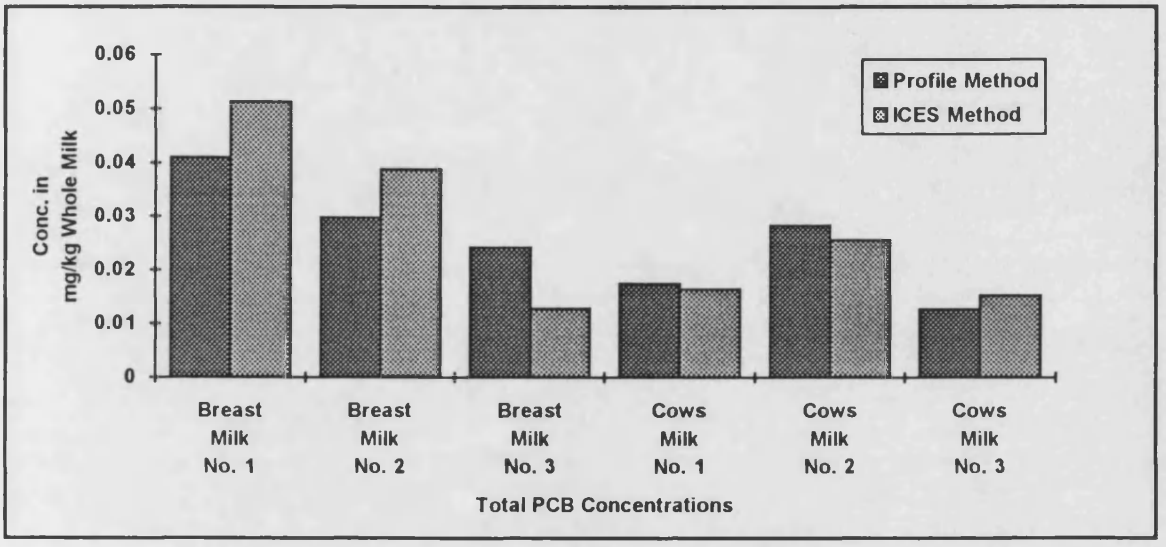


DIAGRAM 8.1: COMPARISON OF TOTAL PCB CONCENTRATIONS OF SIX MILK SAMPLES BY GC-MS USING ICES METHOD & PCB PROFILE METHOD

8.3.2: COMPARISON OF ICES METHOD AND 51 CONGENER METHOD USING GC-MS:

Diagram 8.2 shows the comparison of the Total PCB Concentrations for the six milk samples calculated by the ICES Method and the 51 Congener Method. The diagram shows that the Total PCB results obtained by the two different quantitation methods were similar for all six of the milk samples. The 3 Cows Milk samples all showed higher results when calculated by the 51 Congener Method.

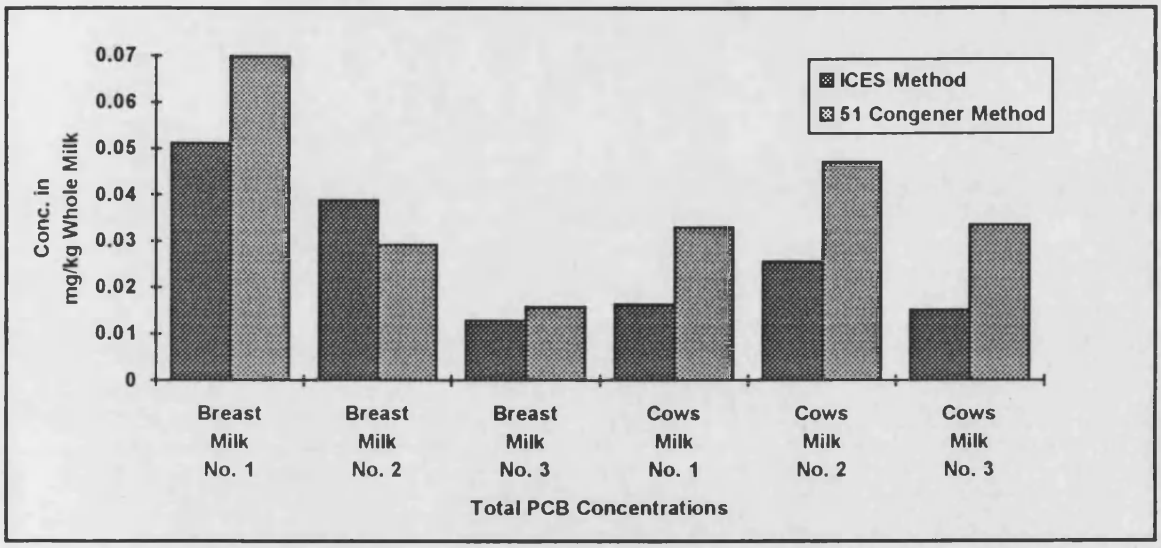


DIAGRAM 8.2: COMPARISON OF TOTAL PCB CONCENTRATIONS OF SIX MILK SAMPLES BY GC-MS USING ICES METHOD & 51 CONGENER METHOD

Diagrams 8.3 and 8.4 show the average concentrations of the 7 individual PCB congeners in the three Breast Milk samples and the three Cows Milk samples, respectively. The 51 Congener Method did not analyse the samples for PCB No. 28, but the results for the other six congeners are compared in the diagrams.

Diagram 8.3 shows the average concentrations in the Breast Milk samples, and it can be clearly seen that the average concentrations of four of the seven PCB congeners by the two different quantitative techniques are similar. The results for PCBs No. 52 and 153 are much higher for the 51 Congener Method than for the ICES Method. The concentrations of the congeners relative to each other show good agreement between the two methods, e.g. the concentration of PCB No. 153 is higher than that of PCB No. 138 which, in turn, is higher than that of PCB No. 180 by both methods. Similarly, the concentrations of the two pentachlorobiphenyl congeners are lower than the other congeners by both methods.

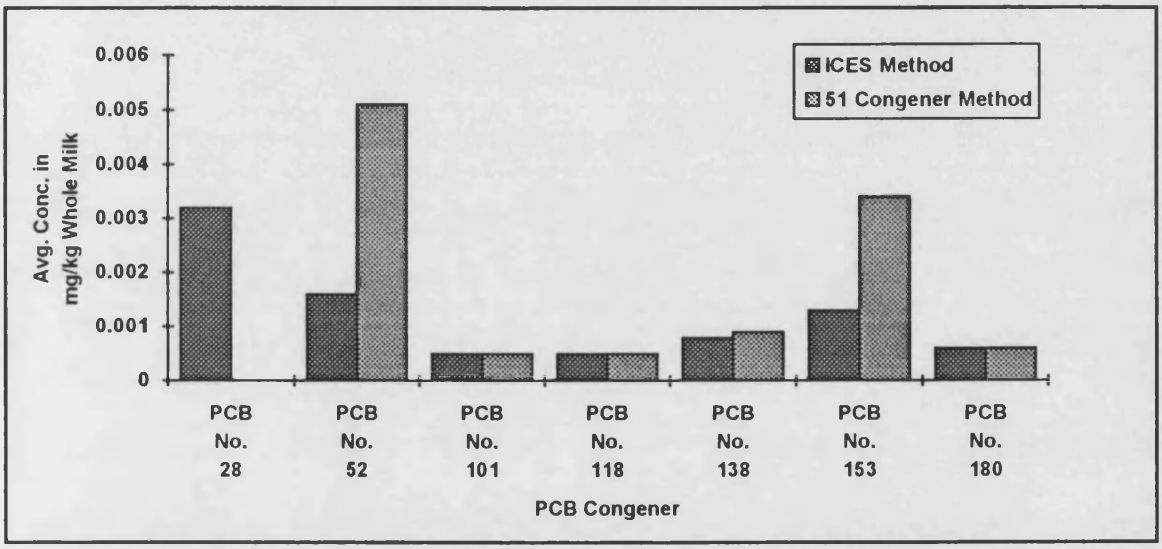


DIAGRAM 8.3: AVERAGE CONCENTRATIONS OF 7 INDIVIDUAL PCB CONGENERS IN BREAST MILK SAMPLES BY ICES METHOD & 51 CONGENER METHOD USING GC-MS

Diagram 8.4 shows the average concentrations of the 7 PCB congeners in the Cows Milk samples. The results show a similar pattern to the Breast Milk results in Diagram 8.3. As in Diagram 8.3, the results for PCBs No. 52 and 153 are much higher for the 51 Congener Method than for the ICES Method. The concentrations of the congeners relative to each other show good agreement between the two methods, just as in Diagram 8.3. Obviously, the fact that PCBs No. 101, 118, and 180 were not detected by either method means that it is difficult to draw any further conclusions from the results.

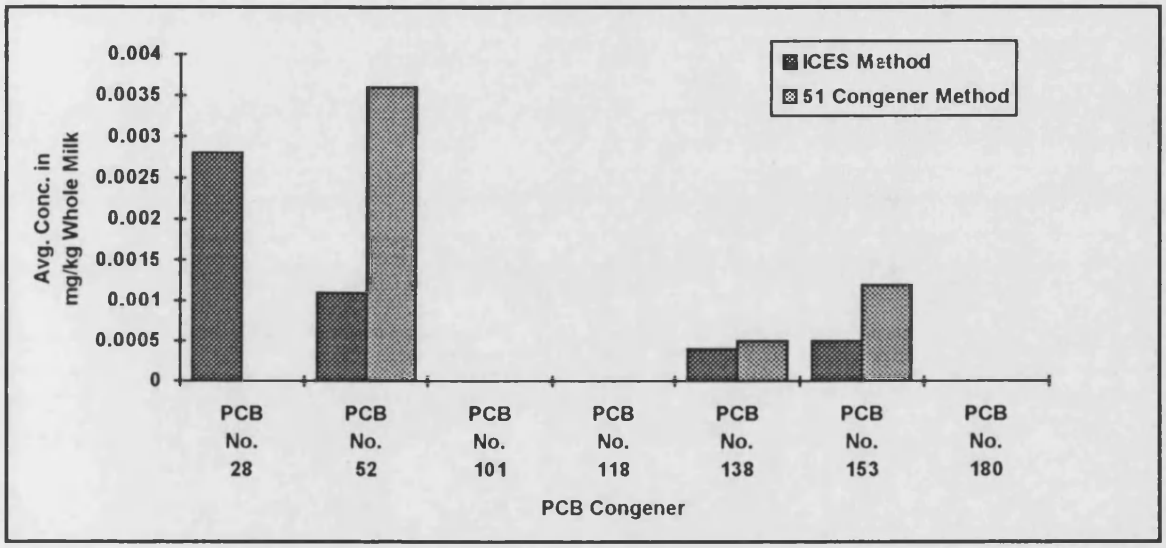


DIAGRAM 8.4: AVERAGE CONCENTRATIONS OF 7 INDIVIDUAL PCB CONGENERS IN COWS MILK SAMPLES BY ICES METHOD & 51 CONGENER METHOD USING GC-MS

8.3.3: COMPARISON OF PCB PROFILE METHOD AND 51 CONGENER METHOD USING GC-MS:

Diagram 8.5 shows the comparison of the Total PCB Concentrations for the six milk samples calculated by the PCB Profile Method and the 51 Congener Method. The Total PCB results calculated by the two different quantitation methods were similar for the six milk samples. As in Section 8.3.2, the results for the three Cows Milk samples were higher when calculated by the 51 Congener Method.

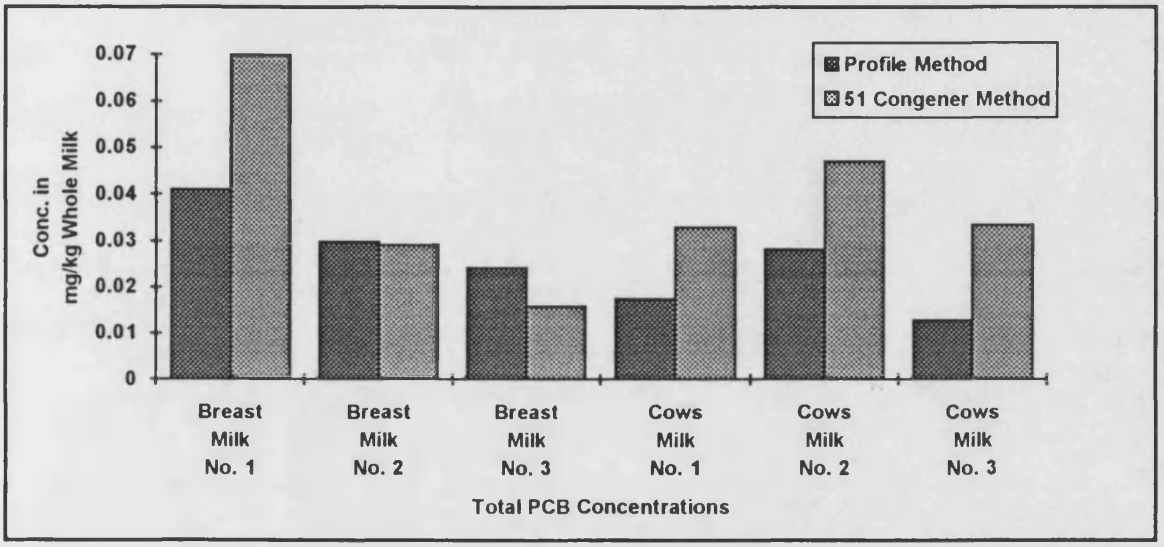


DIAGRAM 8.5: COMPARISON OF TOTAL PCB CONCENTRATIONS OF SIX MILK SAMPLES BY GC-MS USING PCB PROFILE METHOD & 51 CONGENER METHOD

Diagrams 8.6 and 8.7 show the average concentrations of the PCBs by chlorination level in the three Breast Milk samples and the three Cows Milk samples, respectively.

Diagram 8.6 shows the average concentrations in the Breast Milk samples, and it can be clearly seen that the two different quantitation techniques give differing results for the concentrations of PCBs present by chlorination level in the three samples. The major difference between the two techniques can be seen with the trichlorobiphenyls (3Cl), where the result calculated by the 51 Congener Method is three times higher than the result calculated by the PCB Profile Method. The differences between the results for tetra- to heptachlorobiphenyl are less pronounced. The results by chlorination level are mainly higher by the 51 Congener Method, and therefore the differences between results are not caused by the increased number of peaks used in the PCB Profile Method calculations. All of the peaks used in the 51 Congener Method calculations were included in the PCB Profile Method calculations. The discrepancies, therefore, must be caused by differences in the quantitation calculations.

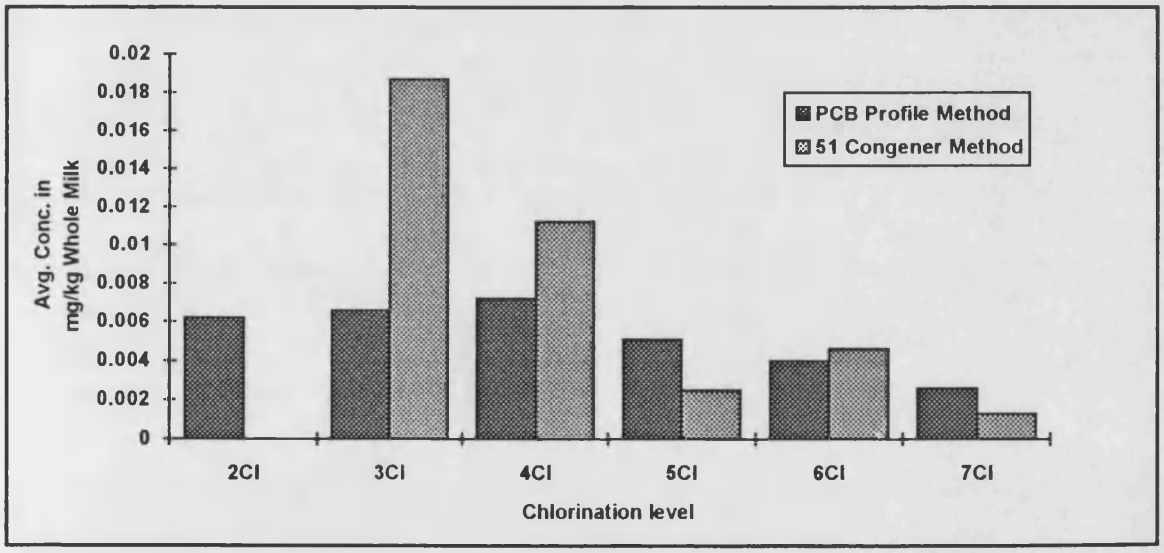


DIAGRAM 8.6: AVERAGE CONCENTRATIONS OF PCBS BY CHLORINATION LEVEL IN BREAST MILK SAMPLES BY PCB PROFILE METHOD & 51 CONGENER METHOD

Diagram 8.7 shows the average concentrations in the Cows Milk samples. The results show a similar pattern to the Breast Milk results in Diagram 8.6, in that the main difference between the two techniques is found in the reported concentrations of trichlorobiphenyls (3Cl). This time the result for the trichlorobiphenyls calculated by the 51 Congener Method is more than four times the result calculated by the PCB Profile Method. The results for the tetra- and hexachlorobiphenyls are in closer agreement.

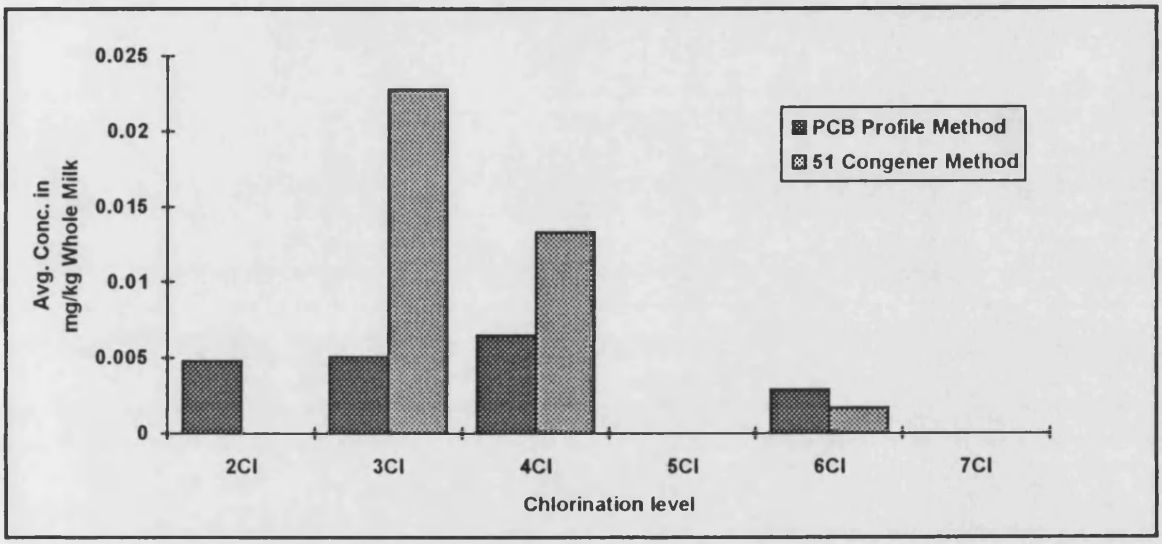


DIAGRAM 8.7: AVERAGE CONCENTRATIONS OF PCBS BY CHLORINATION LEVEL IN COWS MILK SAMPLES BY PCB PROFILE METHOD & 51 CONGENER METHOD

8.3.4: COMPARISON OF ICES METHOD USING GC-MS AND GC-ECD:

A comparison of the Total PCB concentrations for the six milk samples using the two different chromatographic techniques GC-ECD and GC-MS, and the ICES quantitation method is shown in Diagram 8.8. The Total PCB results for four of the six samples, one of the Breast Milk samples and all of the Cows Milk samples, were between 4 and 7 times higher when using the GC-ECD rather than the GC-MS.

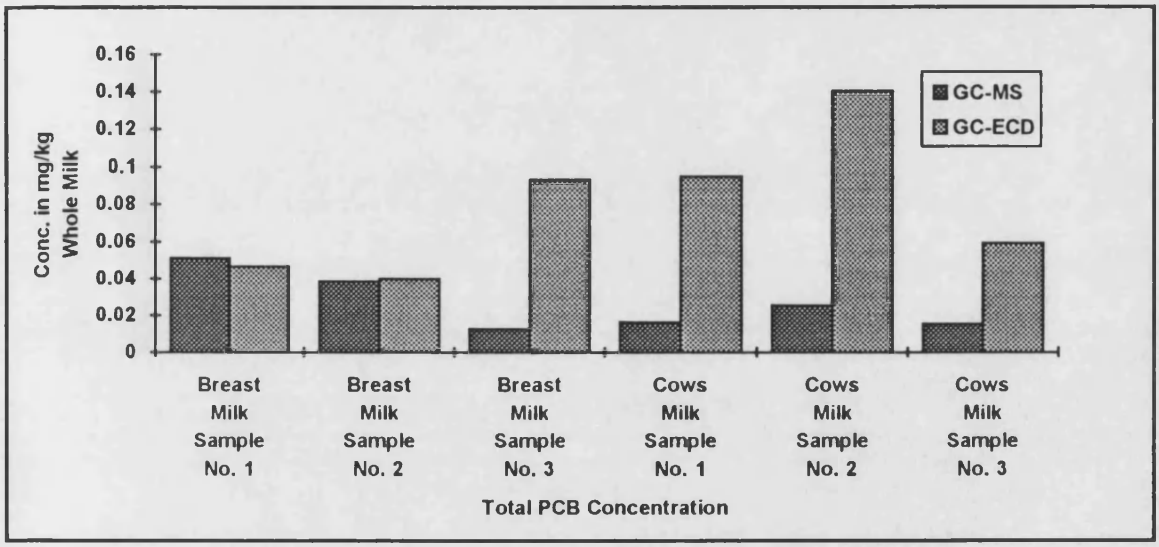


DIAGRAM 8.8: TOTAL PCB CONCENTRATIONS BY ICES METHOD USING GC-ECD AND GC-MS

Diagrams 8.9 and 8.10 show the average concentrations of the 7 individual PCB congeners in the three Breast Milk samples and the three Cows Milk samples, respectively.

Diagram 8.9 shows the average concentrations in the Breast Milk samples, and it can be clearly seen that the average concentrations of five of the seven PCB congeners by the two different chromatographic techniques are similar. However, it is also clear that the results for PCBs No. 28 and, especially, 180 are much higher for the GC-ECD than for the GC-MS. This is almost certainly caused by co-eluting peaks on the GC-ECD which have been screened out by the SIM programme of the GC-MS. This indicates that the co-eluting peaks are not PCBs, but different co-extractants.

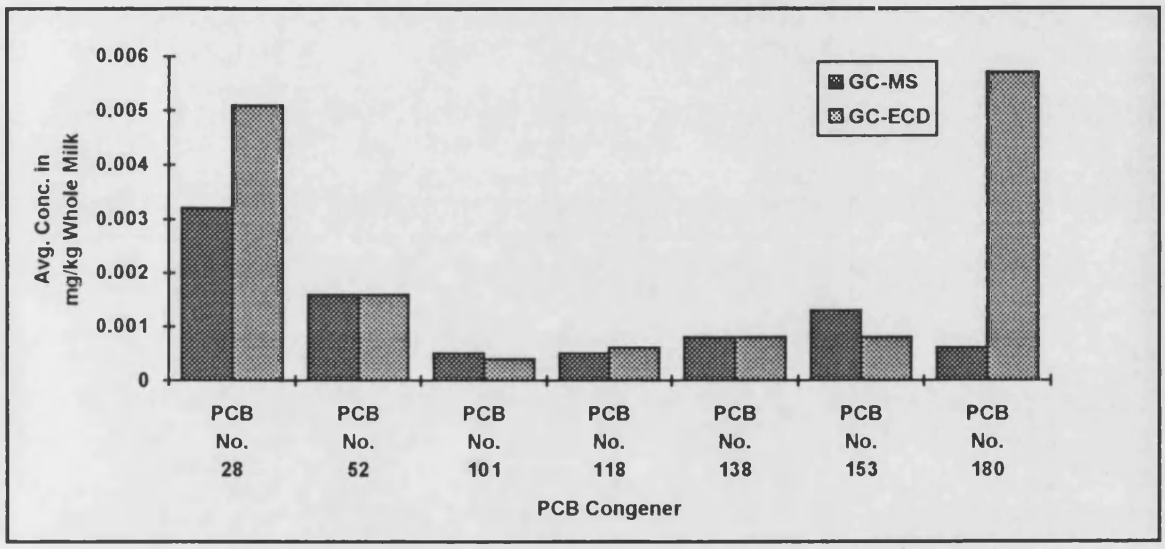


DIAGRAM 8.9: AVERAGE CONCENTRATIONS OF 7 INDIVIDUAL PCB CONGENERS IN BREAST MILK SAMPLES BY ICES METHOD USING GC-MS AND GC-ECD

Diagram 8.10 shows the average concentrations of the 7 PCB congeners in the Cows Milk samples. The results show a similar pattern to the Breast Milk results in Diagram 8.9. The average results for five of the seven congeners are similar for the two chromatographic techniques, while the GC-ECD results for PCBs No. 28 and 180 are much higher than the corresponding GC-MS results. This, again, is almost certainly due to co-eluting non-PCB peaks which are not screened out by the GC-ECD.

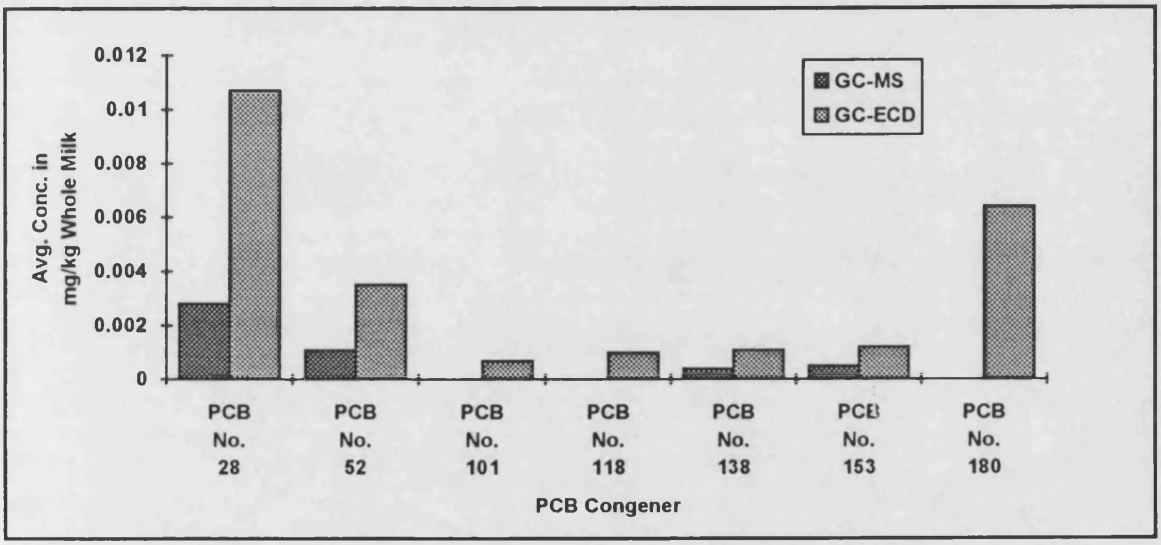


DIAGRAM 8.10: AVERAGE CONCENTRATIONS OF 7 INDIVIDUAL PCB CONGENERS IN COWS MILK SAMPLES BY ICES METHOD USING GC-MS AND GC-ECD

8.3.5: STATISTICAL COMPARISON OF DIFFERENT QUANTITATION TECHNIQUES:

The Total PCB concentrations for the six milk samples have been calculated using four different quantitation techniques, Section 8.2. All of the Total PCB results from Section 8.2 are displayed below in Diagram 8.11.

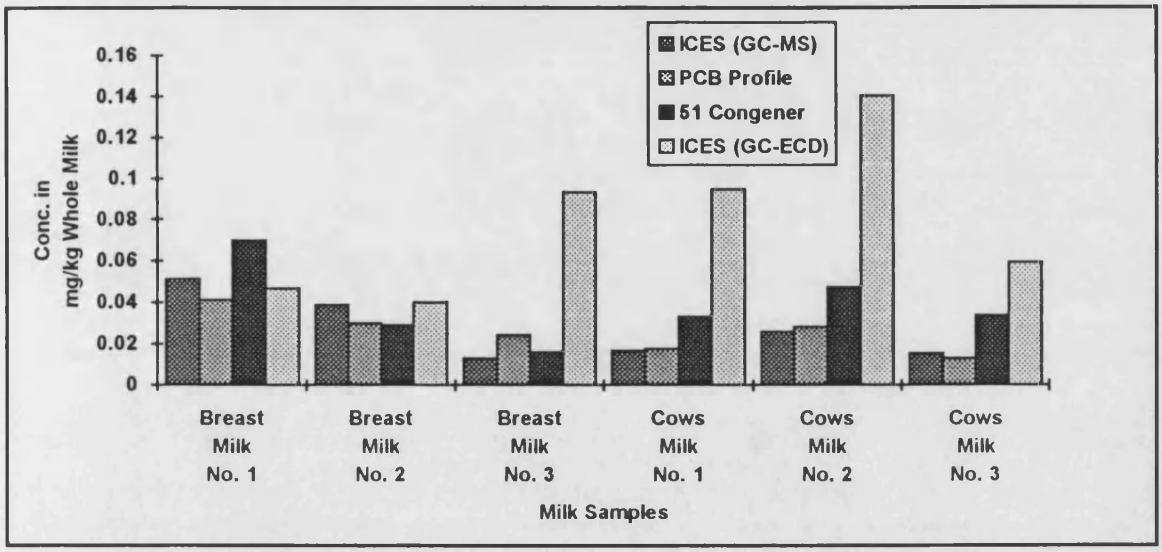


DIAGRAM 8.11: TOTAL PCB CONCENTRATIONS IN THE SIX MILK SAMPLES BY ALL FOUR DIFFERENT QUANTITATION METHODS

The Total PCB results have been tested statistically to determine whether any of the various sample types or quantitation techniques gave rise to significantly different results. In order to decide whether the differences in Total PCB results seen in Diagram 8.11 can be accounted for by random errors, e.g. peak area calculation, a statistical test known as a significance test can be employed. Two-way analysis of variance (ANOVA) was the significance test chosen to investigate the Total PCB results shown in Diagram 8.11.

ANOVA is an extremely powerful statistical technique which can be used to separate and estimate the different causes of variation in a set of results (184). ANOVA can test whether altering the quantitation technique or sample type leads to a significant difference in the Total PCB results obtained. A complicated set of equations is used to calculate the terms needed to determine

the significance of each possible source of variation. The calculations involved are tabulated in the relevant sections below.

The variations caused by differences in sample or sample type, and quantitation technique are calculated. These variations are compared with the calculated residual variation using another significance test, called an F-test (184). This test is used to compare different variations found in a set of data. F is calculated using equation (1):

$$F = s_1^2/s_2^2 \quad - (1)$$

where, s_1^2 & s_2^2 = calculated residual and experimental variances.

The calculated variances are allocated so that the value of F is always greater than or equal to 1. The null hypothesis adopted is that the observed differences in the Total PCB results occurred because of random, i.e. residual, variations. If the calculated value of F is not significantly greater than one, using tables of standard results, then the variations in the Total PCB results are probably caused by random errors and not differences in sample, sample type, or quantitation technique. If the calculated value of F is significantly greater than one, then the differences in the Total PCB results cannot be accounted for simply by random error. In this case, either the difference in samples, or quantitation techniques, or both, are responsible for some of the variations in the Total PCB results observed.

ANOVA was, therefore, used to investigate the way in which the different quantitation techniques, as well as the two different types of sample, affected the Total PCB results. Analysis of variance was also used to determine any possible interactions between the different factors.

8.3.5.1: Comparison Of The Variations In Total PCB Results Caused By Different Samples And Quantitation Techniques Using ANOVA:

The Total PCB results for the six milk samples by the four different quantitation methods are shown in Table 8.6. The row totals and column totals are also shown in Table 8.6, along with a number of other terms needed in the ANOVA calculations detailed below.

Sample Name	Concentration of PCBs in mg/kg Whole Milk					
	Quantitation Method					
	ICES (GC-MS)	PCB Profile	51 Congener	ICES (GC-ECD)	Row Total (T_i)	T_i^2
Breast Milk						
No. 1	0.0512	0.0410	0.0698	0.0468	0.2088	0.0436
No. 2	0.0388	0.0298	0.0291	0.0400	0.1377	0.0190
No. 3	0.0128	0.0242	0.0158	0.0932	0.1460	0.0213
Cows Milk						
No. 1	0.0164	0.0174	0.0329	0.0948	0.1615	0.0261
No. 2	0.0256	0.0282	0.0471	0.1404	0.2413	0.0582
No. 3	0.0152	0.0127	0.0335	0.0592	0.1206	0.0145
Column Total (T_j)	0.1600	0.1533	0.2282	0.4744	1.0159 = T	0.1827 = Total
T_j^2	0.0256	0.0235	0.0521	0.2251	0.3263 = Total	

TABLE 8.6: TOTAL PCB CONCENTRATIONS FOR THE SIX MILK SAMPLES USING FOUR DIFFERENT QUANTITATION TECHNIQUES

The following terms, calculated from Table 8.6, are used in the ANOVA calculations:

r = no. of rows = 6; c = no. of columns = 4; N = r.c = 24; $\Sigma T_i^2 = 0.1827$; $\Sigma T_j^2 = 0.3263$; T = 1.0159; $\Sigma x_{ij}^2 = 0.06493$.

These terms were then used to calculate the factors needed to decide whether there were any significant sources of variation. The mathematical formulae used in these calculations are shown in Table 8.7.

Source of Variation	Sum of Squares	Degrees of Freedom
Between-row	$\Sigma T_i^2/c - T^2/N$	r - 1
Between-column	$\Sigma T_j^2/r - T^2/N$	c - 1
Residual	By subtraction	By subtraction
Total	$\Sigma x_{ij}^2 - T^2/N$	N - 1

TABLE 8.7: FORMULAE FOR TWO-WAY ANOVA

From Miller & Miller (184), pg. 166.

The value for the mean square for each source of variation was calculated by dividing the sum of squares total by the number of degrees of freedom. An F-test was then used to decide whether the mean square values of the various sources of variation differed significantly. A value of F for each source of variation was calculated by dividing the mean square by the residual mean square. The F values obtained were then compared with the published critical values (184) for the given degrees of freedom. These results are shown in Table 8.8.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F	Significant at 5 %
Between-sample	2.675×10^{-3}	5	5.35×10^{-4}	$F_{5,15} = 1.02$	No
Between-treatment	0.01138	3	3.79×10^{-3}	$F_{3,15} = 7.23$	Yes
Residual	7.875×10^{-3}	15	5.25×10^{-4}		
Total	0.02193	23			

TABLE 8.8: RESULTS OF ANOVA CALCULATIONS ON DATA FROM TABLE 8.6

The results in Table 8.8 show that the different milk samples were not a significant source of variation. The value of F calculated for between-sample variation was lower than the critical value of F at the 5 % significance level, for the given degrees of freedom. However, the different quantitation techniques did give rise to significant variations in the Total PCB results obtained. The value of F calculated for between-treatment (i.e. quantitation technique) variation was greater than the critical value of F at the 5 % significance level, for the given degrees of freedom.

8.3.5.2: Comparison Of The Variations In Total PCB Results Caused By Different Sample Types And Quantitation Techniques Using ANOVA:

The next step in discovering the sources of variation in the Total PCB results reported was to analyse the results to determine whether they showed any significant interaction between the particular sample type and quantitation technique employed. Therefore, the six milk samples were split into two groups, the three breast milk samples and the three cows milk samples. This allowed

the results to be checked for possible interactions. The results obtained by grouping the milk samples are shown in Table 8.9, along with a number of terms needed for the calculations.

Sample Name	Concentration of PCBs in mg/kg Whole Milk					
	Quantitation Method					
	ICES (GC-MS)	PCB Profile	51 Congener	ICES (GC-ECD)	Row Total (T_i)	T_i^2
Breast Milk						
Samples	0.0512	0.0410	0.0698	0.0468		
	0.0388	0.0298	0.0291	0.0400	0.4925	0.2426
	0.0128	0.0242	0.0158	0.0932		
Cows Milk						
Samples	0.0164	0.0174	0.0329	0.0948		
	0.0256	0.0282	0.0471	0.1404	0.5234	0.2739
	0.0152	0.0127	0.0335	0.0592		
Column Total (T_j)	0.1600	0.1533	0.2282	0.4744	1.0159 = T	0.5165 = Total
T_j^2	0.0256	0.0235	0.0521	0.2251	0.3263 = Total	

TABLE 8.9: TOTAL PCB CONCENTRATIONS FOR THE THREE BREAST MILK SAMPLES AND THE THREE COWS MILK SAMPLES USING FOUR DIFFERENT QUANTITATION TECHNIQUES

The following terms are used in the calculations:

n = no. of replicates = 3; r = no. of rows = 2; c = no. of columns = 4; $\Sigma T_i^2 = 0.5165$; $\Sigma T_j^2 = 0.3263$; $T = 1.0159$; $\Sigma x_{ijk}^2 = 0.06493$; $\Sigma T_{ij}^2 = 0.1714$.

$C = T^2/n.r.c = 0.0430$.

A slightly different set of equations were used to carry out this comparison of the variations caused by the different sample types and quantitation techniques, and determine any possible interaction. An extra term to calculate the variation of the interaction is included. The equations used for this work are shown in Table 8.10.

Source of Variation	Sum of Squares	Degrees of Freedom
Between-row	$\Sigma T_i^2/n.c - C$	r - 1
Between-column	$\Sigma T_j^2/n.r - C$	c - 1
Interaction	By subtraction	By subtraction
Residual	$\Sigma x_{ijk}^2 - \Sigma T_{ij}^2/n$	r.c.(n - 1)
Total	$\Sigma x_{ijk}^2 - C$	r.c.n - 1

TABLE 8.10: FORMULAE FOR TWO-WAY ANOVA WITH INTERACTION

From Miller & Miller (184), pg. 174.

The mean square values were calculated in the same way as detailed in Section 8.3.5.1. Additionally, an F value was calculated for each source of variation, as well as the interaction term, and the significance of the values tested in the same way. These results are shown in Table 8.11.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F	Significant at 5 %
Between-sample	3.95×10^{-5}	1	3.95×10^{-5}	-	No
Between-treatment	0.01138	3	3.79×10^{-3}	$F_{3,16} = 7.77$	Yes
Interaction	2.71×10^{-3}	3	9.04×10^{-4}	$F_{3,16} = 1.85$	No
Residual	7.80×10^{-3}	16	4.88×10^{-4}		
Total	0.02193	23			

TABLE 8.11: RESULTS OF ANOVA CALCULATIONS ON DATA FROM TABLE 8.9

The variation caused by the difference in sample types was not found to be significant. The between-sample type variation was not significant because the between-sample mean square was less than the residual mean square. This was in agreement with the results in Table 8.6. The variation caused by the different quantitation techniques was found to be significant. The F value found for the between-treatment (i.e. quantitation technique) variation was higher than the critical value of F at the 5 % significance level, for the given degrees of freedom. This agreed, again, with the results from Table 8.6. The additional information provided by calculating an interaction term showed that the interaction between the differences in sample type and quantitation technique was

not significant. The F value calculated for the interaction term was lower than the critical value of F at the 5 % significance level, for the given degrees of freedom.

The statistical calculations above proved that there was no significant interaction between the sample type and the quantitation technique employed. The possible interaction between different sample types and quantitation techniques can be represented graphically. The mean Total PCB values of the three breast milk samples and the three cows milk samples by each of the four different quantitation techniques have been plotted in Diagram 8.12.

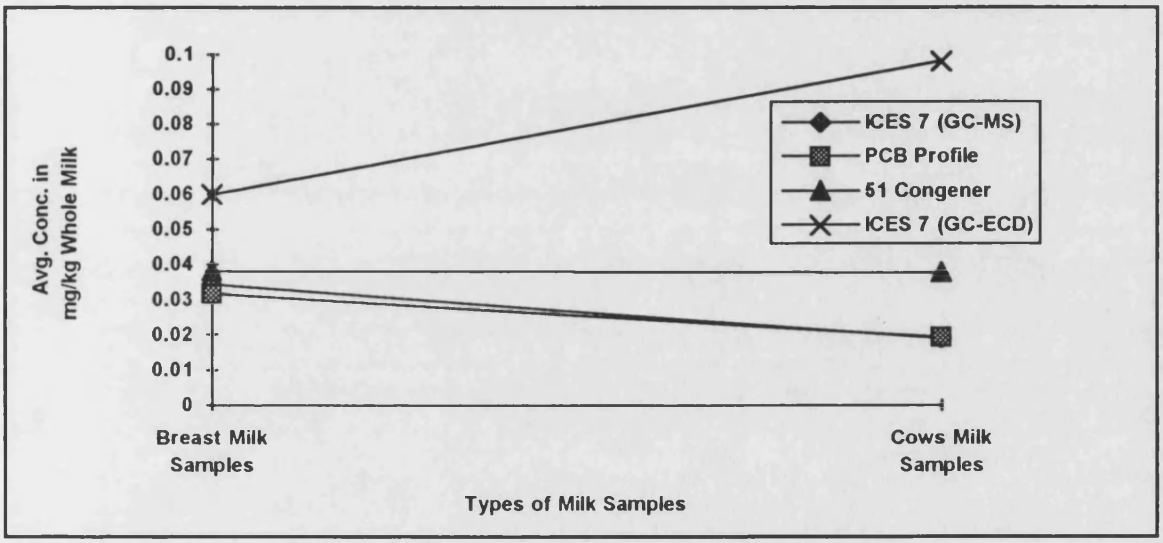


DIAGRAM 8.12: INTERACTION PLOT COMPARING MEAN TOTAL PCB CONCENTRATIONS OBTAINED USING DIFFERENT QUANTITATION TECHNIQUES AND SAMPLE TYPES - TABLE 8.9

In this type of diagram, called an Interaction Plot, parallel curves would indicate no interaction. Diagram 8.12 shows that the curves for the three GC-MS quantitation techniques are close to being parallel, while the curve for the ICES Method by GC-ECD is very different. Therefore, Diagram 8.12 suggests that the GC-ECD quantitation technique seems to be the major cause of variation in the Total PCB results obtained.

The actual Total PCB concentrations obtained by the GC-ECD quantitation technique were also much higher than those obtained by the other three quantitation techniques, Diagrams 8.11 and

8.12. Therefore, the GC-ECD results were removed, and only the Total PCB results obtained by the three different quantitation techniques on the GC-MS were analysed by ANOVA. This would show whether the significant variations in the Total PCB concentrations found when using different quantitation techniques was mainly caused by the large difference between the GC-ECD results and the rest.

8.3.5.3: Comparison Of The Variations In Total PCB Results Caused By Different Sample Types And The GC-MS Quantitation Techniques Using ANOVA:

The next step in discovering the sources of variation in the Total PCB results reported was to analyse the Total PCB results by the three GC-MS quantitation techniques to determine whether they showed any significant variations caused by either the sample type or the quantitation technique employed. The results obtained by the three GC-MS quantitation techniques are shown in Table 8.12, along with a number of terms needed for the calculations.

Sample Name	Concentration of PCBs in mg/kg Whole Milk				
	Quantitation Method				
	ICES (GC-MS)	PCB Profile	51 Congener	Row Total (T_i)	T_i^2
Breast Milk					
Samples	0.0512	0.0410	0.0698		
	0.0388	0.0298	0.0291	0.3125	0.0977
	0.0128	0.0242	0.0158		
Cows Milk					
Samples	0.0164	0.0174	0.0329		
	0.0256	0.0282	0.0471	0.2290	0.0524
	0.0152	0.0127	0.0335		
Column Total (T_j)	0.1600	0.1533	0.2282	0.5415 = T	0.1501 = Total
T_j^2	0.0256	0.0235	0.0521	0.1012 = Total	

TABLE 8.12: TOTAL PCB CONCENTRATIONS FOR THE THREE BREAST MILK SAMPLES AND THE THREE COWS MILK SAMPLES USING THREE DIFFERENT GC-MS QUANTITATION TECHNIQUES

The following terms are used in the calculations:

n = no. of replicates = 3; r = no. of rows = 2; c = no. of columns = 3; $\Sigma T_i^2 = 0.1501$; $\Sigma T_j^2 = 0.1012$; $T = 0.5415$; $\Sigma x_{ijk}^2 = 0.02025$; $\Sigma T_{ij}^2 = 0.05230$.

$C = T^2/n.r.c = 0.01629$.

These terms were then used to calculate the factors needed to decide whether there were any significant sources of variation. The mathematical formulae used in these calculations were those shown previously in Table 8.10.

The mean square values were calculated in the same way as detailed in Section 8.3.5.1. Additionally, an F value was calculated for each source of variation, as well as the interaction term, and the significance of the values tested in the same way. These results are shown in Table 8.13.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F	Significant at 5 %
Between-sample	3.88×10^{-4}	1	3.88×10^{-4}	$F_{1,12} = 1.65$	No
Between-treatment	5.77×10^{-4}	2	2.88×10^{-4}	$F_{2,12} = 1.23$	No
Interaction	1.79×10^{-4}	2	8.93×10^{-5}	-	No
Residual	2.82×10^{-3}	12	2.35×10^{-4}		
Total	3.96×10^{-3}	17			

TABLE 8.13: RESULTS OF ANOVA CALCULATIONS ON DATA FROM TABLE 8.12

The variation in the Total PCB results between sample types was not found to be significant. The value of F calculated for the between-sample variation was lower than the critical value of F at the 5 % significance level, for the given degrees of freedom. This agreed with the results in Section 8.3.5.2. Similarly, the variation caused by interaction between the sample type and the quantitation technique was not found to be significant. The interaction variation was not significant because the interaction mean square was less than the residual mean square.

However, for this set of data, the variation in the Total PCB results between quantitation techniques was not found to be significant. This was the opposite of the results reported in Sections 8.3.5.1 and 8.3.5.2. The value of F calculated for the between-treatment (i.e. quantitation technique) variation was lower than the critical value of F at the 5 % significance level, for the given degrees of freedom. This showed that the significant variation in the Total PCB results caused by the four different quantitation techniques, which was found in Sections 8.3.5.1 and 8.3.5.2, was mainly due to the large difference between the results for the GC-ECD quantitation technique and the results for the three GC-MS quantitation techniques.

8.3.6: SUMMARY OF RESULTS:

The statistical comparison of results in Section 8.3.5 showed that all of the different GC-MS quantitation methods gave similar values for the Total PCB concentrations. However, the results obtained using the ICES Method by GC-ECD were different to those obtained using the different quantitation techniques by GC-MS.

In Section 8.3.5.2, the differences between the Total PCB concentrations obtained by using the four different quantitation techniques were tested. The differences between the Total PCB concentrations were found to be significant at the 5 % significance level.

The differences between the Total PCB concentrations obtained from using the three GC-MS quantitation techniques were tested in Section 8.3.5.3. This time the differences between the Total PCB concentrations were not found to be significant at the 5 % significance level. This proved that the significant variation in the Total PCB results caused by the different quantitation techniques

was mainly due to the large difference between the results for the GC-ECD quantitation technique and the results for the three GC-MS quantitation techniques.

The Total PCB results were much higher by GC-ECD than by GC-MS for four of the six milk samples analysed. Diagrams 8.9 and 8.10 showed that the main reasons for the differences were the higher reported concentrations of PCBs No. 28 and 180 by GC-ECD. These higher concentrations by GC-ECD were almost certainly due to co-eluting peaks which were not PCBs. Such peaks were screened out by the SIM programme used for quantifying the GC-MS results. These findings were in agreement with those of Pavoni *et al.* (72), who also found that concentrations obtained by GC-ECD were systematically higher than those obtained by GC-MS using the same quantitation standard. Pavoni *et al.* (72) also concluded that this descrepancy was caused by the inclusion of extraneous peaks in the GC-ECD results.

The ICES Method provides only the concentrations of 7 selected PCB congeners, by GC-MS (Diagram 8.13), or GC-ECD (Diagram 8.14).

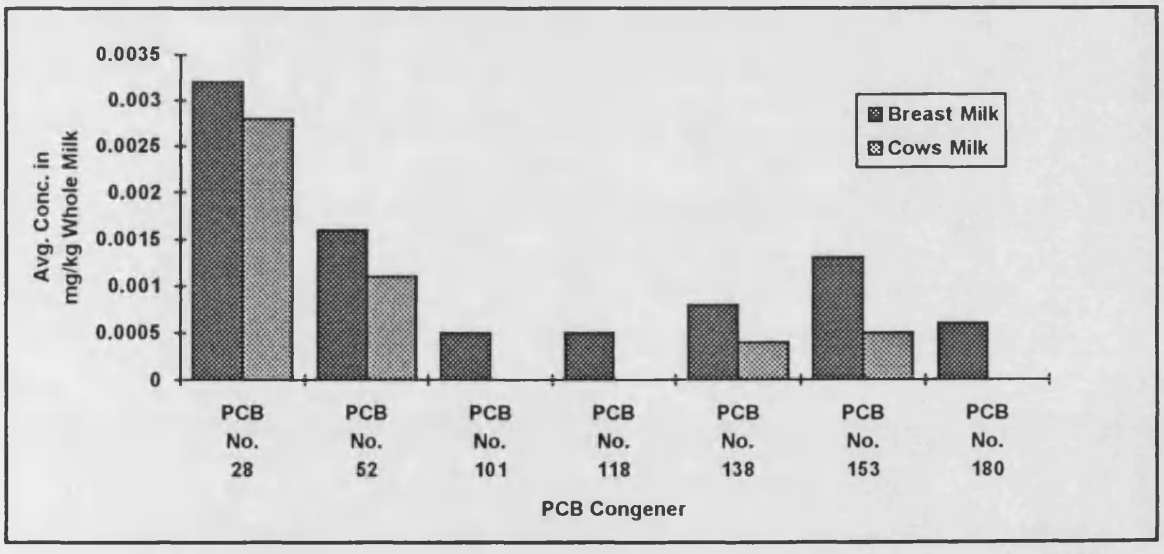


DIAGRAM 8.13: MEAN RESULTS OF 7 INDIVIDUAL PCB CONGENERS OF SIX MILK SAMPLES ANALYSED BY ICES METHOD USING GC-MS

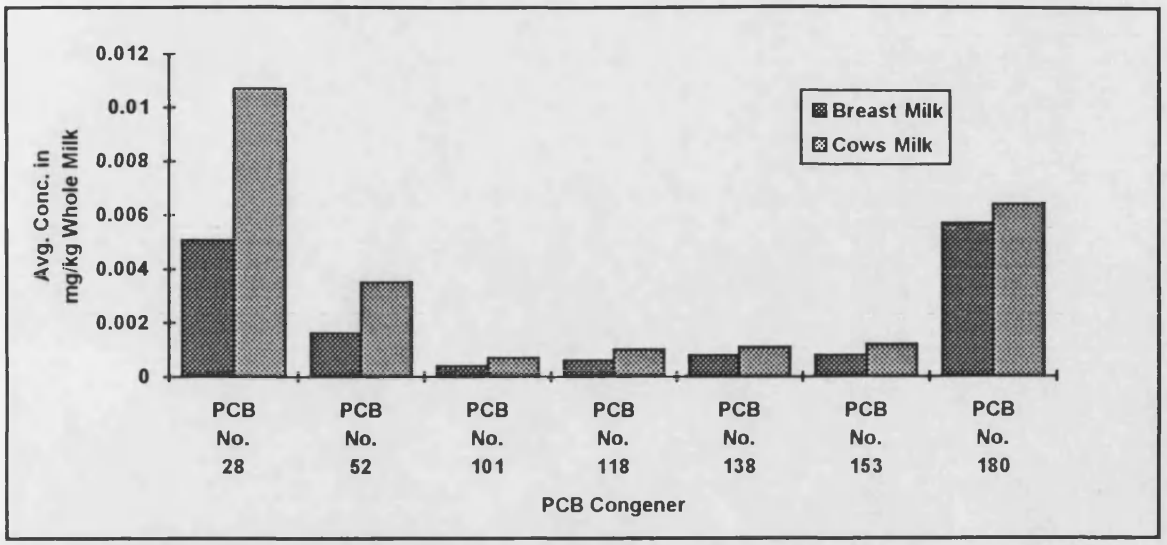


DIAGRAM 8.14: MEAN RESULTS OF 7 INDIVIDUAL PCB CONGENERS OF SIX MILK SAMPLES ANALYSED BY ICES METHOD USING GC-ECD

The above 7 individual PCB congener results can then be used to calculate a Total PCB concentration using a fixed multiplication factor of 4. The multiplication of the 7 congener concentrations by a factor of 4, as used in this work, may not always be appropriate, because the ICES method was originally developed for transformer oils. The 7 PCB congeners chosen represent the most commonly occurring congeners in a range of samples. These congeners are used in German and Dutch legislation on permissable levels in different samples. The ICES Method is, therefore, useful in screening programmes where a cut-off point is set, and the only issue is whether a sample complies with this cut-off point. The ICES Method provides no further information about the nature or possible toxicity of the contamination.

The PCB Profile Method, however, provides more useful information for the analyst than the ICES Method. The results obtained using the PCB Profile Method allow the composition of PCBs to be expressed as a total figure, and also by level of chlorination, Diagram 8.15. The PCB Profile Method quantifies all of the PCB peaks detected in a sample, and allows the analyst to compare the PCBs found in different samples.

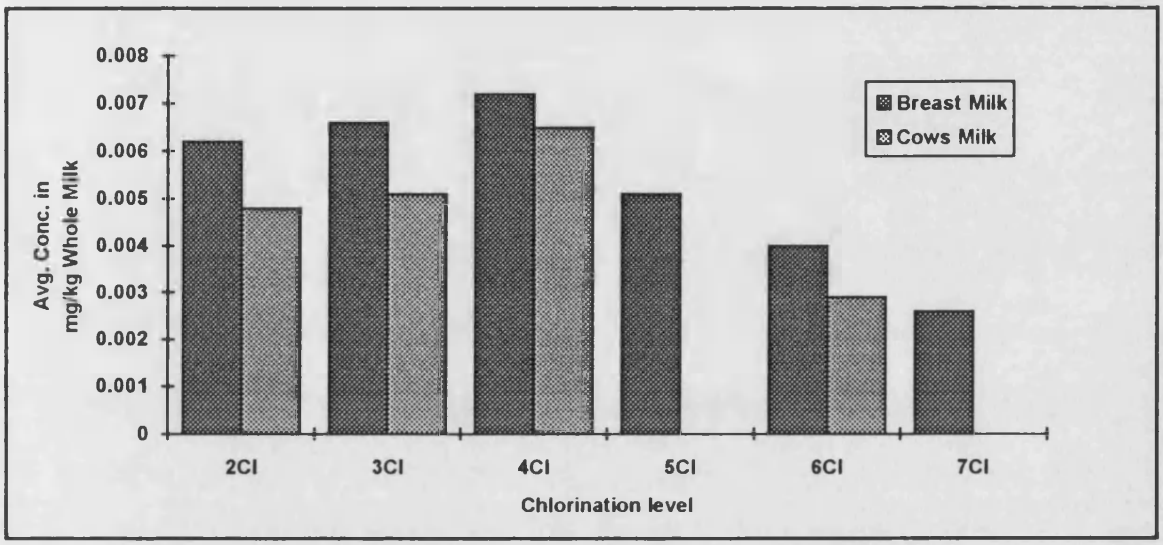


DIAGRAM 8.15: MEAN PCB RESULTS BY CHLORINATION LEVEL OF SIX MILK SAMPLES ANALYSED BY PCB PROFILE METHOD USING GC-MS

The reporting of the results by chlorination level allows the analyst to see whether the PCBs found fit the patterns in other samples or in any of the industrial mixtures, e.g. Aroclors. Any contamination of food samples will be by some sort of industrially used PCB mixture, and such a mixture will contain a large number of individual PCB congeners. The PCB Profile Method quantifies all of the congeners in a food sample.

The PCB Profile Method is designed for use with GC-MS analysis, and utilises the huge amount of data generated by the GC-MS to obtain as much information about the PCBs present as possible. In contrast, the ICES Method is designed for use with GC-ECD analysis, and provides a

quick method of obtaining the minimum information required about the PCBs present, i.e. whether the PCB concentration found is higher or lower than a set level.

The 51 Congener Method allows the PCB results to be looked at by chlorination level, just like the PCB Profile Method, as well as providing additional information on a wide range of individual congeners. The average PCB results by chlorination level obtained by the 51 Congener Method are shown in Diagram 8.16.

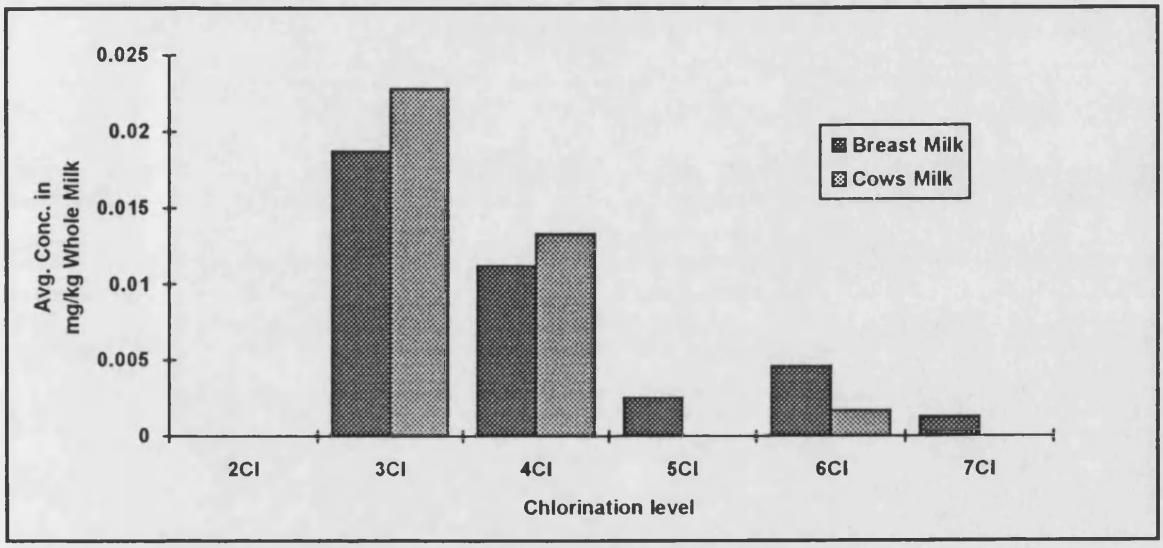


DIAGRAM 8.16: MEAN PCB RESULTS BY CHLORINATION LEVEL OF SIX MILK SAMPLES ANALYSED BY 51 CONGENER METHOD USING GC-MS

The results obtained by the 51 Congener Method can also be used to calculate the levels of each individual PCB congener present in the samples. Previous work carried out by researchers into PCB levels in marine samples led to the selection of the particular 51 congeners used. The congeners selected were the most prevalent ones found in the marine samples and, therefore, provided as much information as possible about the pollution of marine life by industrially produced PCBs. These individual PCB congener results can be used to determine the levels of toxicity in marine samples caused by the contamination.

The 51 congeners are also applicable to other samples, although they were not originally designed for that use. Initially, the four standard mixtures were designed for GC-ECD analyses, and not GC-MS analyses. Therefore, the four standards contain higher concentrations of the lower chlorinated PCB congeners than the higher chlorinated PCB congeners. The ideal GC-MS standard solution would contain higher concentrations of the higher chlorinated PCB congeners. Despite these drawbacks, the Total PCB concentrations calculated by the 51 Congener Method do not differ significantly from those obtained by the other GC-MS quantitation methods.

A number of the 51 congeners, e.g. PCBs No. 52, 101, 118, 138, 153, 180, and 194, have been widely reported as among the most prevalent congeners in a range of different samples (Sections 1.4.3.1 and 1.4.3.2).

The 51 congeners include six of the seven congeners used in the ICES Method, and the individual congener results by the two methods have been compared in Section 8.3.2. Diagrams 8.3 and 8.4 showed that some differences do occur between the concentrations of the individual PCB congeners by the 51 Congener Method and the ICES Method, with the largest differences found for PCBs No. 52 and 153. In addition, there were differences in the PCB concentrations calculated by homologue using the 51 Congener Method and the PCB Profile Method. Diagrams 8.6 and 8.7 showed that the largest differences occurred for the trichlorobiphenyls.

Although there were differences between individual results calculated by the 51 Congener Method and the other GC-MS quantitation techniques, all three methods gave similar Total PCB results for the six milk samples. In addition, the 51 Congener Method provided extra information about the levels of a range of individual PCB congeners in the milk samples, which could be used to determine toxicity levels in the samples.

In conclusion, the work above showed that the three GC-MS quantitation techniques all gave Total PCB concentrations in the six milk samples which were not significantly different. However, the PCB Profile Method and the 51 Congener Method provided more information about the PCBs present in the milk samples than the ICES Method. The ICES Method was originally designed as a quick alternative to more involved quantitation methods like the PCB Profile Method or the use of

individual congeners, but the advent of computerised data handling facilities on both GC-MS and GC-ECD instruments makes most methods similar in terms of the speed of data analysis. The ICES Method is still useful for GC-ECD analyses carried out on instruments without computerised data handling, and in a regulatory context. However, the Total PCB results found by GC-ECD in this work were significantly higher than those found by GC-MS, Section 8.3.5.3. This was caused by non-PCB compounds co-eluting with the PCB congeners, notably PCBs No. 28 and 180, Diagrams 8.9 and 8.10. The possible problem of incomplete sample clean-up must be addressed before GC-ECD analysis can be used for PCB analysis.

The PCB Profile Method is the method commonly used for GC-MS analyses, and provides a large amount of information about the nature of the contamination in a sample. The 51 Congener Method involves more calculations than the PCB Profile Method. It is used for GC-MS analyses when additional information about a wide range of individual PCB congeners in a sample is required, and in situations where the toxicity levels in a sample are of importance.

CHAPTER 9: CONCLUSIONS

The ubiquity of PCB contamination in a number of British foodstuffs and breast milk samples has been demonstrated by the work presented in Chapters 3, 4, 5, and 6. All of the samples analysed in these chapters were found to contain PCBs at quantifiable levels. The amount of research on the levels of PCB contamination in Great Britain that has been reported in the scientific literature is much lower than in a number of other countries, e.g. Canada and the U.S.A. Although the Welsh Office has recently published research on the levels of PCBs in air, soil, and grass samples taken from near to a commercial incineration plant (29), more analyses of a wider range of samples will be necessary for a full appreciation of the levels and possible problems caused by PCB contamination within Great Britain.

Although the analysis of PCBs has not previously enjoyed a high priority in Great Britain, the increasing influence of the European Union on domestic environmental policy will mean that PCB analyses will be legally required in a range of different commercial activities. An example of this is in the recently privatised water industry. Whilst drinking water is most unlikely to contain detectable amounts of PCBs, the increasingly important areas of waste management and land reclaimation will require comprehensive analytical examination.

In addition, because of the current economic climate, the water companies are looking to simplify the routine analytical methods that are employed. This can only be achieved by using fewer analytical methods and instruments that are dedicated to a narrow group of compounds, and by developing methods and instruments that are capable of measuring as many compounds as possible. The new generation of GC-MS instruments can provide the enormous amount of information that the industry requires. The increased sensitivity over earlier GC-MS instruments, by a factor of approximately ten, and the affordability of the current GC-MS models should ensure that these take over from specialist instruments e.g. GC-ECD.

The increased routine use of GC-MS will provide the analyst with a vast amount of data on each sample. The analyst must then decide how much of this GC-MS data is of use. For example,

in Chapters 3, 5, and 6 the Total PCB results have been determined by homologue in a number of different samples. This data can, then, highlight differences in the PCB contamination found in various samples.

For example, Diagram 3.3 showed the average Total PCB concentrations in the Fish Liver Oil and Fish Oil samples analysed in Chapter 3. There was a wide difference between the PCB patterns found in the Fish Liver Oil and Fish Oil samples. The Fish Liver Oil results showed higher concentrations of the pentachloro and hexachlorobiphenyls indicating that the PCBs stored in the liver had been metabolised. The higher chlorinated PCB congeners metabolise more slowly than the lower chlorinated ones, and, therefore, have remained unaltered in the liver of the fish. In contrast, the Fish Oil samples showed a PCB pattern like that of Aroclor 1242. These samples were extracted from blended whole fish samples.

The Cows Milk samples analysed in Chapter 5 contained higher concentrations of the lower chlorinated PCB congeners, and all of the samples analysed contained roughly the same pattern of congeners, similar to Aroclor 1242. This can be seen in Diagram 5.2.

In contrast to the Cows Milk samples, the 23 Breast Milk samples analysed in Chapter 6 showed three completely different PCB patterns, reflecting the differences in the amount of metabolism of PCBs by the individual mothers. Examples of the three distinct PCB patterns can be seen in Diagram 6.3. The mother only secretes milk after giving birth, so that any contaminants present will have been metabolised to some degree, depending on the individual women.

All PCB congeners are metabolised by higher animals, as reported by Erickson (1), page 39. The higher the level of chlorination, the slower the rate of metabolism. The metabolism of PCB congeners must be taken into consideration when analysing results from samples such as breast milk, for example.

Good accuracy and precision of results for PCBs present at very low levels can be difficult to achieve. In Section 5.2.2.1 it was found that the analysis of one cows milk sample ten times gave a relative standard deviation (RSD) of 17.6 %, with the original analysis method described. However, by using close visual inspection of the chromatograms as well as mathematical testing to

decide which peaks to quantify, an improvement in the precision of the PCB results was achieved over the method of simply using mathematical testing. The use of visual inspection gave an RSD of 2.0 % for one sample analysed six times. Additionally, it was found that the improvement in precision was greater for the homologues with lower actual PCB concentrations. This illustrated that close visual inspection by the analyst can help achieve a greater degree of precision for concentrations of PCBs near to the limit of quantitation.

The advances in analytical methodology are being matched in the experimental methods now being used. Improvements are currently beginning to be made in both the extraction and clean-up methods used for different samples. For many years classical laboratory methods such as Soxhlet extraction and column chromatography were used. These were time-consuming, labour intensive, and used large volumes of toxic organic solvents. In recent years, these classical methods have begun to be superceded by the use of supercritical fluid technology. Some initial investigations into the use of Supercritical Fluid Extraction (SFE) and Supercritical Fluid Chromatography (SFC) for PCB analysis have been detailed in Chapter 7. The JASCO system that was used, donated by Mettler-Toledo Ltd., Halstead, G.B., was found to be extremely easy to operate. Obviously, a lot more research is needed to fully understand the properties of supercritical fluids, and to develop and optimise techniques for individual samples. However, the initial investigations carried out into the use of supercritical fluid techniques proved highly encouraging.

In all of the work in Chapters 3, 4, 5, and 6, sample preparation involved the use of Soxhlet extraction overnight, followed by solvent volume reduction with a Kuderna-Danish apparatus, and a final clean-up using an HPLC technique. All of this, coupled with the actual GC-MS analysis, meant that an average of only six samples could be analysed each week. The use of the supercritical fluid system meant that a sample could be extracted within 15 - 20 minutes, and cleaned-up in under 30 minutes. This implies that the same six samples which would take a week to prepare for analysis using classical extraction methods, could be ready for analysis within one working day using the supercritical fluid apparatus. This massive time saving is allied to the reductions in solvent volumes and glassware achieved with the supercritical fluid apparatus.

Supercritical fluid techniques can, therefore, achieve an increase in sample throughput over traditional extraction techniques.

Obviously, the sample throughput for routine analyses is vitally important, and any technique that helps to increase throughput will find wide application in the commercial and regulatory sectors. Supercritical fluid techniques are also less labour intensive than the currently used methodology. In addition, throughout the work reported in Chapter 7 the apparatus proved to be robust and reliable. A wide range of companies now produce dedicated supercritical fluid systems, which can extract a number of samples simultaneously, further increasing sample throughput. Therefore, the increasing availability and affordability of supercritical fluid equipment certainly points to the future widespread use of this technique for a range of applications in day-to-day laboratory work.

As well as the advances in the extraction and analytical methodology of samples for PCB analysis, there have also been improvements in the quantitation techniques employed. There are now a number of different PCB standards and quantitation methods that can be used which allow the analyst to get the precise information that is required. A number of these different standards and quantitation methods were compared in Chapter 8.

A simple PCB screening technique involving the measurement of seven selected PCB congeners was detailed in Section 8.1.1.1. This can be used for regulatory purposes, when the full quantitation of samples below the regulatory cut-off point is of no interest. It was shown that this technique could be used to estimate Total PCB concentrations, and that these were not significantly different to those obtained using rather more involved quantitation techniques. Therefore, in cases where the only information required is whether or not a sample contains a Total PCB concentration higher or lower than a set limit, a simple screening technique such as the ICES Method, is perfectly adequate. This technique does not however give any additional information about the PCB contamination found. For example, the toxicological implications of the contamination and the original source are not dealt with. In addition, a screening method fails to fully utilise the data

provided by a GC-MS analysis, and simply ignores a large amount of the data in order to reach a quick final result.

The use of individual PCB congeners as standards enables the analyst to properly harness the huge amount of useful data generated by a GC-MS analysis. The analyst may choose to use a commercially available PCB congener mixture, or prepare a standard mixture *in situ*. This allows the analyst to fine tune the analysis to provide exactly the data required. For example, the analyst may be interested in the possible source of the contamination, in which case a method such as that detailed in Section 8.1.1.2 can be used. The PCB Profile Method provides Total PCB results determined separately for each homologue. This PCB Profile Method can be used to match by chlorination level the PCBs found in a sample to the PCBs found in, for example, an Aroclor.

Alternatively, by selecting a set of individual PCB congeners, an analyst can generate information on the possible biological and toxicological impact of the PCB contamination. In Section 8.1.1.3 a method involving 51 PCB congeners was detailed. These 51 congeners were selected from previous analyses of marine samples. The standard solutions containing the 51 congeners were originally designed for the quantitation of marine samples on a GC-ECD. However, despite the fact that the standards were not ideal, Total PCB concentrations were obtained, using the 51 congeners, for milk samples using a GC-MS. These Total PCB concentrations were not significantly different to those obtained by the ICES Method or the PCB Profile Method. There were differences between some of the individual congener results obtained by the ICES Method and the 51 Congener Method, and between some of the homologue results obtained by the PCB Profile Method and the 51 Congener Method. Overall these differences were minor, and could probably be reduced with some fine tuning of the quantitation techniques. As well as providing Total PCB concentrations, the individual results for the 51 congeners could then be used to determine the toxicological implications of the PCBs found in the milk samples.

The 51 Congener Method used in Chapter 8 is not the only individual congener method reported, and other examples of this type of approach have been used. McFarland & Clarke (20) selected a set of 36 congeners which were either prevalent in the environment, preferentially

bioaccumulated, or potentially toxic. Steinwandter (70) selected 21 non-ortho and mono-ortho substituted congeners for analysis in breast milk samples.

Therefore, different sets of PCB congeners are recommended by various groups for the analysis of a range of different samples. However, most of these different sets contain a fair number of congeners which have been found in many different samples, e.g. PCBs No. 101, 138, 153, and 180. There are a number of these different standard sets of PCB congeners that are commercially available. Alternatively, by purchasing individual congeners, a homemade PCB mixture can be prepared. A quick search of the scientific journals before an analysis should provide enough information to select which PCB congeners should be of interest.

The levels of the non-ortho substituted PCB congeners can also be found by using the right quantitation standard. These are the most toxic PCB congeners, but are usually present at lower levels than many other congeners. Mes & Weber (44), Tanabe *et al.* (85), and Van Rhijn *et al.* (106) have all determined the levels of non-ortho substituted PCB congeners in a range of samples. The results obtained can then be assessed for toxicological importance by using Toxic Equivalency Factors (TEFs), as outlined by Kannan *et al.* (185). The use of TEFs led Dewailly *et al.* (132) to conclude that PCBs represented a higher risk than PCDDs or PCDFs in samples of breast milk.

PCBs have been found in a massive range of samples worldwide, and the levels found do not suggest an end to the problem of PCB contamination in the short term. This is despite the discontinuation of commercial PCB production. Huge quantities of PCBs remain in the environment, and the risks of leakage from older electrical equipment still exist. Therefore, the analysis of samples for PCBs will continue to be important, and it is up to each analyst to provide quick, accurate, and relevant results by careful selection of appropriate extraction, analytical, and quantitation methods.

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No.	Substitution pattern	No.	Substitution pattern	No.	Substitution pattern	No.	Substitution pattern
1	2	53	2,2',5,6'	105	2,3,3',4,4'	157	2,3,3',4,4',5'
2	3	54	2,2',6,6'	106	2,3,3',4,5	158	2,3,3',4,4',6
3	4	55	2,3,3',4	107	2,3,3',4',5	159	2,3,3',4,5,5'
4	2,2'	56	2,3,3',4'	108	2,3,3',4,5'	160	2,3,3',4,5,6
5	2,3	57	2,3,3',5	109	2,3,3',4,6	161	2,3,3'.4.5'.6
6	2,3'	58	2,3,3',5'	110	2,3,3',4',6	162	2,3,3',4',5,5'
7	2,4	59	2,3,3',6	111	2,3,3',5,5'	163	2,3,3',4',5,6
8	2,4'	60	2,3,4,4'	112	2,3,3',5,6	164	2,3,3',4',5',6
9	2,5	61	2,3,4,5	113	2,3,3',5',6	165	2,3,3',5,5',6
10	2,6	62	2,3,4,6	114	2,3,4,4',5	166	2,3,4,4',5,6
11	3,3'	63	2,3,4',5	115	2,3,4,4',6	167	2,3',4,4',5,5'
12	3,4	64	2,3,4',6	116	2,3,4,5,6	168	2,3',4,4',5',6
13	3,4'	65	2,3,5,6	117	2,3,4',5,6	169	3,3',4,4',5,5'
14	3,5	66	2,3',4,4'	118	2,3',4,4',5	170	2,2',3,3',4,4',5
15	4,4'	67	2,3',4,5	119	2,3',4,4',6	171	2,2',3,3',4,4',6
16	2,2',3	68	2,3',4,5'	120	2,3',4,5,5'	172	2,2',3,3',4,5,5'
17	2,2',4	69	2,3',4,6	121	2,3',4,5',6	173	2,2',3,3',4,5,6
18	2,2',5	70	2,3',4',5	122	2',3,3',4,5	174	2,2',3,3',4,5,6'
19	2,2',6	71	2,3',4',6	123	2',3,4,4',5	175	2,2',3,3',4,5',6
20	2,3,3'	72	2,3',5,5'	124	2',3,4,5,5'	176	2,2',3,3',4,6,6'
21	2,3,4	73	2,3',5',6	125	2',3,4,5,6'	177	2,2',3,3',4',5,6
22	2,3,4'	74	2,4,4',5	126	3,3',4,4',5	178	2,2',3,3',5,5',6
23	2,3,5	75	2,4,4',6	127	3,3',4,5,5'	179	2,2',3,3',5,6,6'
24	2,3,6	76	2',3,4,5	128	2,2',3,3',4,4'	180	2,2',3,4,4',5,5'
25	2,3',4	77	3,3',4,4'	129	2,2',3,3',4,5	181	2,2',3,4,4',5,6
26	2,3',5	78	3,3',4,5	130	2,2',3,3',4,5'	182	2,2',3,4,4',5,6'
27	2,3',6	79	3,3',4,5'	131	2,2',3,3',4,6	183	2,2',3,4,4',5',6
28	2,4,4'	80	3,3',5,5'	132	2,2',3,3',4,6'	184	2,2',3,4,4',6,6'
29	2,4,5	81	3,4,4',5	133	2,2',3,3',5,5'	185	2,2',3,4,5,5',6
30	2,4,6	82	2,2',3,3',4	134	2,2',3,3',5,6	186	2,2',3,4,5,6,6'
31	2,4',5	83	2,2',3,3',5	135	2,2',3,3',5,6'	187	2,2',3,4',5,5',6
32	2,4',6	84	2,2',3,3',6	136	2,2',3,3',6,6'	188	2,2',3,4',5,6,6'
33	2',3,4	85	2,2',3,4,4'	137	2,2',3,4,4',5	189	2,3,3',4,4',5,5'
34	2',3,5	86	2,2',3,4,5	138	2,2',3,4,4',5'	190	2,3,3',4,4',5,6
35	3,3',4	87	2,2',3,4,5'	139	2,2',3,4,4',6	191	2,3,3',4,4',5',6
36	3,3',5	88	2,2',3,4,6	140	2,2',3,4,4',6'	192	2,3,3',4,5,5',6
37	3,4,4'	89	2,2',3,4,6'	141	2,2',3,4,5,5'	193	2,3,3',4',5,5',6
38	3,4,5	90	2,2',3,4',5	142	2,2',3,4,5,6	194	2,2',3,3'.4,4',5,5'
39	3,4',5	91	2,2',3,4',6	143	2,2',3,4,5,6'	195	2,2',3,3',4.4'.5.6
40	2,2',3,3'	92	2,2',3,5,5'	144	2,2',3,4,5',6	196	2,2',3,3',4,4',5',6
41	2,2',3,4	93	2,2',3,5,6	145	2,2',3,4,6,6'	197	2,2',3,3',4,4',6,6'
42	2,2',3,4'	94	2,2',3,5,6'	146	2,2',3,4',5,5'	198	2,2',3,3',4,5,5',6
43	2,2',3,5	95	2,2',3,5',6	147	2,2',3,4',5,6	199	2,2',3,3',4,5,6,6'
44	2,2',3,5'	96	2,2',3,6,6'	148	2,2',3,4',5,6'	200	2,2',3,3',4,5',6,6'
45	2,2',3,6	97	2,2',3',4,5	149	2,2',3,4',5',6	201	2,2',3,3 ',4',5,5',6
46	2,2',3,6'	98	2,2',3',4,6	150	2,2',3,4',6,6'	202	2,2',3,3',5,5',6,6'
47	2,2',4,4'	99	2,2',4,4',5	151	2,2',3,5,5',6	203	2,2',3,4,4',5,5',6
48	2,2',4,5	100	2,2',4,4',6	152	2,2',3,5,6,6'	204	2,2',3,4,4',5,6,6'
49	2,2',4,5'	101	2,2',4,5,5'	153	2,2',4,4',5,5'	205	2,3,3',4,4',5,5',6
50	2,2',4,6	102	2,2',4,5,6'	154	2,2',4,4',5,6'	206	2,2',3,3',4,4',5,5',6
51	2,2',4,6'	103	2,2',4,5',6	155	2,2',4,4',6,6'	207	2,2',3,3',4,4',5,6,6'
52	2,2',5,5'	104	2,2',4,6,6'	156	2,3,3',4,4',5	208	2,2',3,3',4,5,5',6,6'
						209	2,2',3,3',4,4',5,5',6,6'

APPENDIX 1

SYSTEMATIC NUMBERING OF PCB CONGENERS INTRODUCED BY BALLSCHMITER AND ZELL (5) AND ACCEPTED BY IUPAC